Crop and nutrient harvest indices for spring wheat genotypes grown with different fertiliser and carbon dioxide levels, under field and controlled environments.

A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Doctor of Philosophy

at

Lincoln University

New Zealand

by

Emmanuel Chakwizira

Lincoln University

Canterbury, New Zealand

2020

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Historically, genetic gains in wheat (*Triticum aestivum* L.) yield have been attributed to breeding advances which resulted in increased crop harvest index (CHI: the ratio between harvested grain and total above-ground biomass), and improved agronomic practices, such as better nitrogen (N) fertiliser management. In this study, two experiments were carried out in the field in 2017—2018 (Experiment 1), and in a glasshouse in 2018—2019 (Experiment 2). These were used to quantify the CHI, nutrient harvest indices (nitrogen, NHI and other nutrients, NuHIs) and N use efficiency (NUE: ratio between grain yield and amount of N supplied)) for six spring wheat genotypes ('Discovery', 'Duchess' 'Reliance', PFR-2021, PFR-3019 & PFR-3026) grown at low and optimum N fertiliser supply. Then two controlled environment experiments (3 and 4) were used to quantify the CHI, NHI and NuHIs for 'Discovery' under ambient and elevated carbon dioxide (CO_2) ($aC0_2$ and eCO_2; respectively), at low and optimum phosphorus (P) and potassium (K) fertiliser supply. The aims of this study was to investigate the influence of fertiliser supply and growth environment on crop growth, nutrient accumulation, partitioning and harvest indices of spring wheat genotypes.

Overall, the CHI values depended on carbon remobilisation to the grain, but there was no relationship between CHI and grain yield. 'Duchess' had a lower CHI (0.33±0.04) than the average of the other genotypes (0.56±0.01; 0.44±0.04) in Experiments 1 and 2, respectively. This was explained by its lower translocation of straw biomass to the grain component, as reflected in its low thousand-grain weight (TGW, g) and high screenings. The lower overall CHI in Experiment 2 (~0.42±0.03), compared with 0.50—0.55 in Experiments 1, 3 and 4,

could have been caused by high temperature episodes near anthesis, which resulted in reduced number of grains per unit area (grain density). The CHI was also reduced by P deficiency, which reduced the area of individual leaves (carbon source) and number of fertile tillers (carbon sink), hence the low above-ground biomass (AGB) and grain yield.

In all four experiments, the relationship between TGW and grain density, showed that the highest yielding genotype 'Discovery' was positive and above the regression line. This higher TGW for 'Discovery' was due to greater total AGB, because it remobilised the same proportion of total carbon to the grains as the other genotypes. These results confirm that the relationship between TGW and grain density can be used to explain yield differences.

The greater AGB and grain yield in Experiments 1 and 2, for 'Discovery' resulted from the fastest pre-anthesis leaf area expansion rate, higher maximum green leaf area index (GLAI) and longer GLA duration above the critical GLAI during the grain-filling period, which resulted in greater intercepted radiation. In contrast, all genotypes had a conservative specific leaf N (SLN) content above the critical threshold of 1.1 g N/m^2, which meant their photosynthetic capacity and therefore the radiation use efficiency were not different. Furthermore, in Experiments 3 and 4, the optimally fertilised crops had larger flag leaf area and more fertile tillers per tube, thus a higher leaf area that lead to higher intercepted radiation. The AGB for these crops increased by 11—23% with eCO_2 and grain yield increased by 6—14%. The AGB decreased by ~90.0%, from 57.4±1.28 g/tube for the optimally fertilised crops to 5.29 g/tube for the P deficient crops.

The NHI (ratio between N accumulated in the grain and N accumulated in the AGB at harvest maturity) differences among the genotypes were small, ≤ 6.40% in Experiments 1 and 2, and lower for 'Discovery' and 'Duchess', compared with the other genotypes. However, NHI was severely reduced (37%) by P deficiency compared with the optimally fertilised crops. The NuHIs (ratio between nutrients accumulated in the grain and nutrients accumulated in the AGB at harvest maturity) differed among the genotype across the experiments, but were inconsistent among genotypes in the different environments. There was no relationship between NuHIs and the proportion of nutrients at anthesis. Therefore, individual NuHIs were a function of remobilisation efficiency, rather than timing of nutrient uptake. High NHI and PHI across the environments, were due to their efficient translocation from the vegetative to the grain component, while the low CaHI and KHI showed these

nutrients were not readily translocated to the grain, with 60—100% of Ca, K, N and P having been accumulated by anthesis. There was a strong, positive relationship between grain N concentration and grain sulphur (S) and zinc (Zn) concentration, and a negative relationship to grain K concentration in Experiments 1 and 2. This has implications for human health, with increased concentration of S and Zn, being a positive result.

In Experiments 1 and 2, NUE differed among the genotypes at optimum N fertiliser supply, higher for 'Discovery' compared with 'Duchess' and 'Reliance'. Similar NUE at low N fertilier supply shows that the selected genotypes had no differentiating traits that could be used for breeding, specific to low N fertility conditions. This was despite fact that these genotypes were recommended by wheat breeders on the basis that they had a range of attributes that could enhance NUE. In Experiment 1, NUE was explained by the N uptake efficiency (NupE) at both N fertiliser rates, while in Experiment 2, NUE was explained by the N utilisation efficiency (NutE). These inconsistent results meant that NUE was not a stable trait, and therefore is limited as a criterion for future breeding selection.

The contribution of this research to future breeding was premised on the confirmation that CHI has plateaued at ~0.50. Results suggest that further grain yield increases must come from genetic enhancements that either increase total AGB at the current CHI levels, or with increased CHI, as some genotypes had higher CHI values of up to 0.59. The effects of N and P fertiliser supply on CHI, NHI and NuHI highlights the importance of fertiliser management on wheat production, and could be used alongside breeding to increase grain yield. At the agronomic level, the insights from this research can improve our understanding of the importance of P fertiliser on yield. At the research level, such understanding shows the physiological mechanisms to inform breeding and improve predictive models of wheat production. In this study, CHI did not respond to increasing CO_2 level. However, response of CHI to increasing CO_2 level has been reported, under water or N stress. Therefore, the interaction of P fertiliser supply, water and/ or N stress needs to be investigated in future.

Keywords: *Triticum aestivum* **L.**, aboveground biomass (AGB), ambient carbon dioxide (aCO_2), anthesis, crop harvest index (CHI), elevated CO_2 (eCO_2), harvest maturity, nitrogen harvest index (NHI), nutrient harvest index (NuHI), physiological traits.

Acknowledgements

I would like to thank my excellent supervisory team of Professor Derrick Moot, Associate Professor Mitchell Andrews and Dr Edmar Teixeira, who provided guidance and support through my studies and whose open door policy ensured that the program progressed on schedule. It was a privilege to have your advice and extensive expertise. I really appreciate your tireless work on those thesis chapter drafts, and the discussions around them.

This remarkable project was completed under The New Zealand Institute for Plant & Food Research Limited's (Plant & Food Research) Sustainable Agro-ecosystems (SAE) programme, with funding from the Strategic Science Investment Fund (SSIF). I would also like to acknowledge the technical assistance from various teams at Lincoln University and Plant & Food Research. Lincoln University teams, capably led by Brent Richards (Greenhouse Nurseries), Stuart Larsen (Biotron facility), Malcom Smith (Dryland Pastures Research) and Roger Cresswell (Lincoln University Laboratory), and Plant & Food Research teams led by Shane Maley (Science Team Leader - Field Operations, Cropping Systems & Environment) and Chris Dunlop (Laboratory Manager - Cropping Systems & Environment). To Dr Paul Johnston (Pre-Breeding team) and Catherine Munro (Annual Crops team) at Plant & Food Research, thank you for the fruitful discussions and email messages on wheat genotypes to help me understand breeding aspects relevant to New Zealand.

To my 15-year-old daughter, Miranda, who helped in the field experiment, and was a source of inspiration and encouragement, thank you very much. I hope I can be of use to you when you take similar challenges in the future. I also want to thank everyone who crossed my path during these last three years, and all the magnificent people who contributed to this research project. I am grateful and honoured that you have been part of this journey.

Declaration

I was involved in all aspects of the experiments described in this dissertation, including field sample collection, laboratory measurements, statistical analyses, and the drafting and final edits of this manuscript.

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Abbreviations

Abbreviation	Description	Units
aCO_2	Ambient carbon-dioxide	µmol/mol
AFR	Apparent fertiliser N recovery	%
AGB	Above ground biomass	t/ha
AGN	Above ground nitrogen	kg/ha
CaHI	Calcium harvest index	kg/kg
CO_2	Carbon-dioxide	µmol/mol
CuHI	Copper harvest index	g/g
DM	Dry matter	t/ha
DW	Dry weight	%
eCO_2	Elevated carbon-dioxide	µmol/mol
DAE	Days after sowing	-
d.f.	Degrees of freedom	-
EC	Emulsifiable concentrate	-
FAR	Foundation for Arable Research	-
FeHI	Iron harvest index	g/g
FSC	Flowable suspension concentrate	-
FLS	Flag leaf size	cm^2
GD	Grain density	-
GLAI	Green leaf area index	cm^2/ cm^2
$GLAI_{crit}$	Critical green leaf area index	cm^2/ cm^2
GPC	Grain protein concentration	%
GS	Growth stage	-
Ht	Height	cm
ICP–OES	Inductively Coupled Plasma Optical Emission Spectrometry	-
IPAR	Incident photosynthetically active radiation	MJ/m^2
IPCC	Intergovernmental Panel on Climate Change	-
KHI	Potassium harvest index	kg/kg
LAER	Leaf area expansion rate	m^2/m^2
LASR	Leaf area senescence rate	m^2/m^2

LL	Leaf length	cm
LSD	Least significance rate	-
LTM	Long term mean	-
LW	Leaf width	cm
Loc. cit	'Loco citato' or 'in the place cited'	-
K	A form factor, in the calculation of flag leaf area	-
MgHI	Magnesium harvest index	g/g
MnHI	Manganese harvest index	g/g
N_g	Grain nitrogen	kg/ha
N_g%	Grain nitrogen concentration	%
NHI	Nitrogen harvest index	kg/kg
NOAA	National Oceanic and Atmospheric Administration	-
NP	Neutron probe	-
NR	Nitrogen remobilisation	kg/ha
NuR	Nutrient remobilisation	kg/ha
NRE	Nitrogen remobilisation efficiency	%
NuRE	Nutrient remobilisation efficiency	%
NUE	Nitrogen use efficiency	kg/kg
NIWA	National Institute of Water and Atmospheric Research	-
NuHI	Nutrient harvest index	-
NupE	Nitrogen uptake efficiency	kg/kg
NutE	Nitrogen utilisation efficiency	kg/kg
O_2	Oxygen	-
PANU	Post anthesis nitrogen uptake	kg/ha
PANuU	Post anthesis nutrient uptake	kg/kg
PFR	Plant & Food Research Limited	-
PGG-Wrightson	Pyne Gould Guinness and Wrightson Limited	-
PHI	Phosphorus harvest index	kg/kg
R_{acc}	Accumulated daily intercepted IPAR	MJ/m2
Rht	Reduced height	-
RUE	Radiation use efficiency	g/MJ

SC	Soluble concentrate	-
SHI	Sulphur harvest index	kg/kg
T_a	Average daily temperature	°C
T_b	Base temperature	°C
TDR	Time domain reflectometer rods	-
TGW	Thousand grain weight	g
Tt_{acc}	Accumulated thermal time	°Cd
UK	United Kingdom	-
USDA	United States Department of Agriculture	-
v.r.	Variation ratio	-
WDG	Water disposable granule	-
WS	Water soluble	-
ZnHI	Zinc harvest index	g/g

Equations

Chapter 1: General Introduction

1.1 Global wheat production

Globally, wheat is the most widely grown crop, and its production has risen 3.4-fold from 222 million tons (~1.10 t/ha) in 1961 to >750 million tons (~3.40 t/ha) in 2018 (FAOSTAT 2019; Toreti et al. 2019) (Figure 1.1 A). This has been attributed to improved agronomic and breeding advances (Donald & Hamblin 1976; Austin et al. 1980;; Craigie et al. 2015).

From a breeding perspective, the development of semi-dwarf genotypes that are characterised by lodging-resistance (Donald 1968; Austin et al. 1989; Berry et al. 2007), and increased crop harvest index (CHI: the ratio between harvested grain and total aboveground biomass (AGB)) (Donald 1962; Donald & Hamblin 1976)) were the key attributes for increased grain yield. Semi-dwarf genotypes enabled the use of higher rates of synthetic nitrogen (N) fertilisers (Austin et al. 1980), resulting in increased grain yields and the subsequent high CHI. Currently, more than 50% of the world's population are fed as a result of the use of synthetic fertiliser (Ritchie 2017). The CHI values reported over the last 40 years indicates that it has plateaued at ~0.50 (Austin et al. 1980; Gifford et al. 1984; Austin 1999; Berry et al. 2007; Dai et al. 2016; Senapati & Semenov 2019). This means further yield increases must come from genetic enhancements that increase total biomass production (Calderini et al. 1995b; Austin 1999; Berry et al. 2007), while utilizing resources as efficiently as possible.

The concept of CHI has been extended to partitioning of nutrients (e.g. nitrogen harvest index, NHI: the ratio between N accumulated in the grain and N accumulated in the AGB at harvest maturity)) (Sinclair 1998) and has provided a range of responses whose implications for production and breeding can be explored (Hay 1995). The NHI for wheat has been extensively reported in historical (Austin et al. 1977; Desai & Bhatia 1978; Austin et al. 1980) and recent (Andersson & Johansson 2006; Gorjanović et al. 2011; Frels et al. 2018) studies. There have also been recent reports of nutrient harvest indices (NuHIs) for other macro- and micro-nutrients, mainly for winter wheat (Hamnér et al., 2017), while the few reports for spring sown wheat are dated (Miller et al., 1993; Hocking, 1994). The current study will determine NuHIs for modern spring wheat genotypes, and compare those with established values and winter wheat.

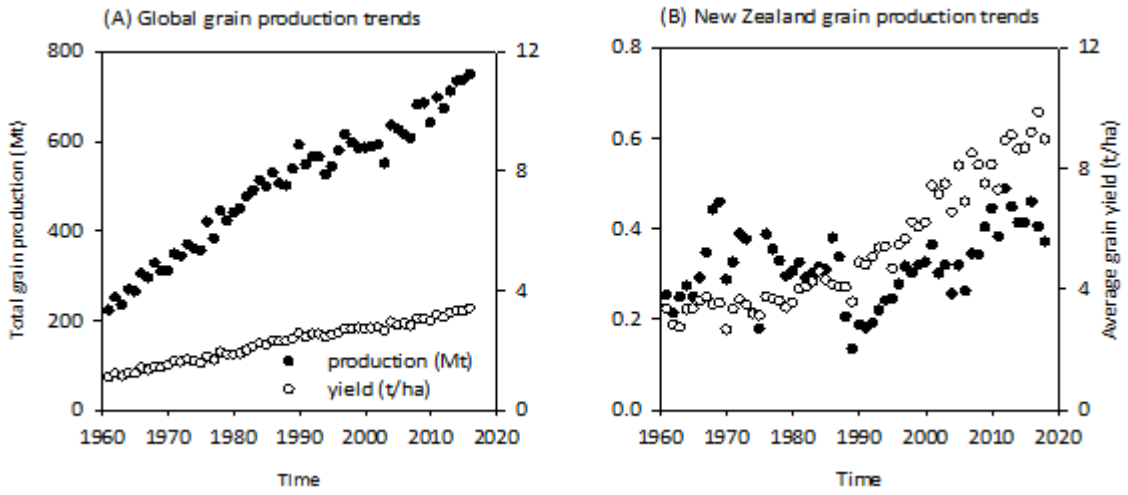

Figure 1.1: Trends in: (A) global and (B) New Zealand wheat total grain production (Mt; ●) and average grain yield (t/ha; ○); 1961 to 2017. Source: Computed by author based on FAOSTAT online database, 30 Dec. 2019 (http://www.fao.org/faostat/en/#data/QC).

In New Zealand, wheat is the second most grown crop after potatoes; with total annual production of ~0.46 million tons (Figure 1.1 B). Grain yields of 12.0-16.0 t/ha have been reported for winter wheat (Armour et al., 2004; Craigie et al., 2015), with average yields of ~ 9.0 t/ha. Grain yields of 8.0—10.0 t/ha have been reported for spring-sown wheats (AIMI 2018; Dawson et al. 2018; Michel et al. 2018). These yields are higher than the global average of ~ 3.4 t/ha (Figure 1.1 A; Hawkesford et al. 2013; Hawkesford 2014) and have been made possible by a combination of improved genetics, suitable environment and efficient crop management techniques. The management techniques include the application of N fertilisers (Craigie et al. 2015; Michel et al. 2018), appropriate sowing dates and use of agrichemicals for crop protection (Poole et al. 2013). Successive Guinness World Records for wheat grain yields have been achieved in New Zealand, most recently with 16.8 t/ha in 2017 and 17.4 t/ha in 2020 (Anonymous 2020). These are within the reported simulated potential yields of 17.0—20.0 t/ha for New Zealand and UK conditions (Mitchell & Sheehy 2018; Senapati & Semenov 2019). The current average yields in New Zealand are ~52% of record values, showing the potential yield gap to be explored.

1.2 Nitrogen (N) fertiliser

In New Zealand, applications of up to 200 kg N/ha to wheat crops are common (Craigie et al. 2015; Dawson et al. 2018; Michel et al. 2018). These amounts are expected to avoid N deficiency to produce and maintain a green canopy area capable of intercepting and utilising

solar radiation to optimise biomass production, grain growth and quality. These N fertiliser rates are consistent with those reported internationally, for high yielding wheat crops (Austin 1999; Barraclough et al. 2010; Hawkesford 2014). Inadequate N supply affects growth through the rate of leaf area expansion, and consequently green leaf area (Lawlor et al. 1989). Nitrogen also influences the rate of canopy photosynthesis by directly affecting the quantity of catalytic enzymes (e.g. Rubisco) (Lawlor et al. 1989; Adam et al. 2000) and indirectly through a reduced leaf area for radiation interception (Muchow & Davis 1988). The N status of a crop can be determined using the specific leaf N (SLN, g N/m^2 leaf) (Sinclair & Horie 1989) and the threshold for wheat is 1.1 g N/m^2 leaf (Meinke et al. 1998); derived from a range of 0.8—1.3 g N/m^2 leaf reported previously (Sinclair & Horie 1989; Meinke et al. 1997). The SLN content below the threshold reduces the photosynthetic capacity and hence radiation use efficiency (RUE; g/MJ).

The excessive use of synthetic fertilisers has been associated with negative environmental impacts in agricultural systems (Ali et al. 2018). Therefore, there is motivation to search for a balance between high economic yields and minimum environmental impacts. Such needs prompted plant breeders to try to develop new genotypes with high N use efficiency (NUE; the ratio of grain yield to total amount of N available) (Moll et al. 1982), through the identification of physiological traits that can be used in future breeding selections.

1.3 Environmental influences

The principal environmental factors that affect plant growth and development, in the absence of water stress, are solar radiation and temperature, respectively (Ritchie et al. 1998). However, the balance between carbon dioxide (CO_2) and oxygen (O_2) (Bloom 2006) is also important as this controls the level of photosynthetic carbon assimilation or photorespiration in C_3 plants such as wheat. Growth responses of plants to elevated atmospheric CO_2 (eCO_2) (Sections 2.6 & 6.1) are directly influenced by other factors, such as water or nutrient availability (Taub 2010). The increased growth rates of CO_2 enriched plants are bound to create high demand for mineral nutrients that make nutrient availability particularly important. Most studies on effects of eCO_2 on plant production have been done under optimal nutrient conditions (Manderscheid et al. 1995; Li et al. 2007) or has focused on N (Conroy 1992; Pettersson & McDonald 1994; Bloom et al. 2002; Bloom et al. 2010). Therefore, the effect of phosphorus (P) and potassium (K) deficiencies on growth and

nutrient partitioning of wheat are little known (Section 2.6). Further to determine the effects of genotype and N fertiliser supply on CHI, NHI, NuHIs and NUE, this study will investigate the influence of CO_2, P and K level on CHI and NuHIs for spring wheat.

1.4 Genotypes selection and characterisation

The six spring wheat genotypes used in this study (Appendix 1.1) were recommended by New Zealand wheat breeders to have a range of attributes that could enhance NUE (Johnston, Paul., pers comm.). They were 'Discovery', 'Duchess', 'Reliance', PFR-2021, PFR-3019 and PFR-3026. The three named genotypes are milling wheats currently on the New Zealand seed market, while the numbered genotypes/ lines (e.g. PFR-2021) are not released, but are used as breeding material within The New Zealand Institute for Plant & Food Research (Plant & Food Research; PFR), as they have traits perceived to enhanced NUE. Milling wheat genotypes in New Zealand are classified as 'premium' (e.g. 'Reliance' and 'Duchess') which have total grain protein content (GPC) of 12.0 -13.5% or as 'milling'/'medium' (e.g. 'Discovery') whose GPC tends to be lower at 11.0-12.5% (FAR, 2019; Munro, Catherine., pers.comms). The differing GPC is either due to: total N uptake (Rodgers & Barneix 1988) or translocation of N to the grain (Van Sanford & MacKown 1986) or (3) post-anthesis N uptake or a combination of these factors (Dhugga & Waines 1989). These attributes could also result in different grain yields and consequently NHI (Appendix 1.1) and NUE (Section 2.3.2) among the genotypes.

1.5 Aims and objectives

This study aims to understand how crop growth, nutrient accumulation, partitioning and harvest indices of spring sown wheat genotypes are influenced by nutrient supply and growth environment. The two objectives of this study are to determine the effects of:

1. Genotype, N fertiliser supply and their interaction on CHI, NHI, NuHIs and NUE for six spring sown wheat genotypes reported to have different NUE. This objective was tested in Experiment 1 (field; 2017—2018) and Experiment 2 (glasshouse; 2018—2019) (Chapters 3—5).

2. Carbon-dioxide (CO_2) level (ambient [aCO_2]; elevated [eCO_2]), P and K fertiliser supply and their interactions on CHI, NHI and NuHIs for spring sown wheat, cv. 'Discovery'. This objective was tested in Experiments 3 and 4 (growth chambers; 2019—2020) (Chapter 6).

Growth and development in Experiment 1 were determined six times during the growing season, with particular emphasis on anthesis and harvest maturity. Growth and nutrient uptake levels for Experiments 2, 3 and 4 were determined at anthesis and harvest maturity. Support measurements, such as canopy development (e.g. green leaf area index), number of fertile tillers, flag leaf area and plant height will be determined as a way of explaining the treatment effects on CHI, NHI, NuHIs and NUE.

1.5.1 Thesis structure

This thesis consists of seven chapters as illustrated in Figure 1.2. This General Introduction (Chapter 1) is followed by a Review of Literature (Chapter 2), before the description of Experiment 1 (Chapters 3-4) that used six genotypes to compare crop growth and development, and nutrient uptake and partitioning. Experiment 2 (Chapters 5) was used to confirm genotype rankings on CHI, NHI, NUE, NuHI and temporal nutrient uptake pattern from Experiment 1, before Experiment 3 and 4 (Chapter 6) which focussed on CHI, NHI, NuHI and temporal nutrient uptake pattern responses to CO_2, P and K levels. The General Discussion (Chapter 7) integrates the key findings to interpret results in relation to current knowledge and summarises knowledge gaps across chapters for suggested future research. Each chapter contains an introduction, materials and methods, results, discussion and a summary with main conclusions.

Specifically;

- Chapter 1: General Introduction provides an overview of global and New Zealand wheat production, and the impacts of management and environmental conditions on growth and development.
- Chapter 2 presents a review of literature on spring (and winter) wheat crop and nutrient harvests indices (CHI & NuHIs, respectively) in the context of the potential mechanisms for increased biomass and grain yield for different genetic materials and the effects of management (e.g. N stress). Nutrient accumulation and partitioning are reviewed as they relate to growth, biomass accumulation and grain yield.
- Chapter 3: Field experiment (Experiment 1), presents results on biomass and grain yield, and N uptake and use efficiency of the six spring wheat genotypes grown with low (0 kg/ha) or optimum (200 kg/ha) N fertiliser supply. Optimum fertiliser rate was

determined from 'Sirius' Wheat Calculator (Section 3.2.2.2). This chapter provides the underlying framework for subsequent experimental chapters in this thesis.

- Chapter 4: Field experiment, presents the temporal macro- and micro-nutrient uptake patterns, accumulation and translocation of the different spring sown wheat genotypes grown at low and optimum N fertiliser supply**.** As the biomass, grain yield and N dynamics differed among genotypes and between N fertiliser supplies, an assessment of the responses of the whole suite of macro-and micro-nutrients was also performed to establish relationships to N fertiliser supply and genotypes, and the implications for grain quality (NuHI).

- Chapter 5: Glasshouse experiment (Experiment 2) was used to confirm the results of Chapters 3 and 4, and genotype rankings achieved in Experiment 1. In this section, relationships between N and other nutrients established in Experiment 1 are confirmed for the vegetative and reproductive stages, as well as the grain component for all six genotypes.

- Chapter 6: Growth chamber experiment (Experiments 3 and 4). Having established the effects of genotypes and N fertiliser supply on CHI, NHI, NuHIs and NUE, further investigation of the influence of environment and other macro-nutrient deficiencies were carried out. These experiments investigated the effect of CO_2 level, and P and K fertiliser rate on CHI, NHI and NuHIs. The highest yielding wheat genotype from Experiments 1 and 2, 'Discovery' was grown under different P and K levels, at ambient and elevated CO_2 levels in Experiments 3 and 4.

- Chapter 7: General Discussion**,** integrates findings from four experiments, investigated how CHI, NHI, NuHIs and NUE was affected by genotype, N fertiliser supply, CO_2 level, and P and K fertiliser supply. Chapter 7 also provides specific recommendations for future breeding effort, in relation to grain yield and quality and hence food nutrition.

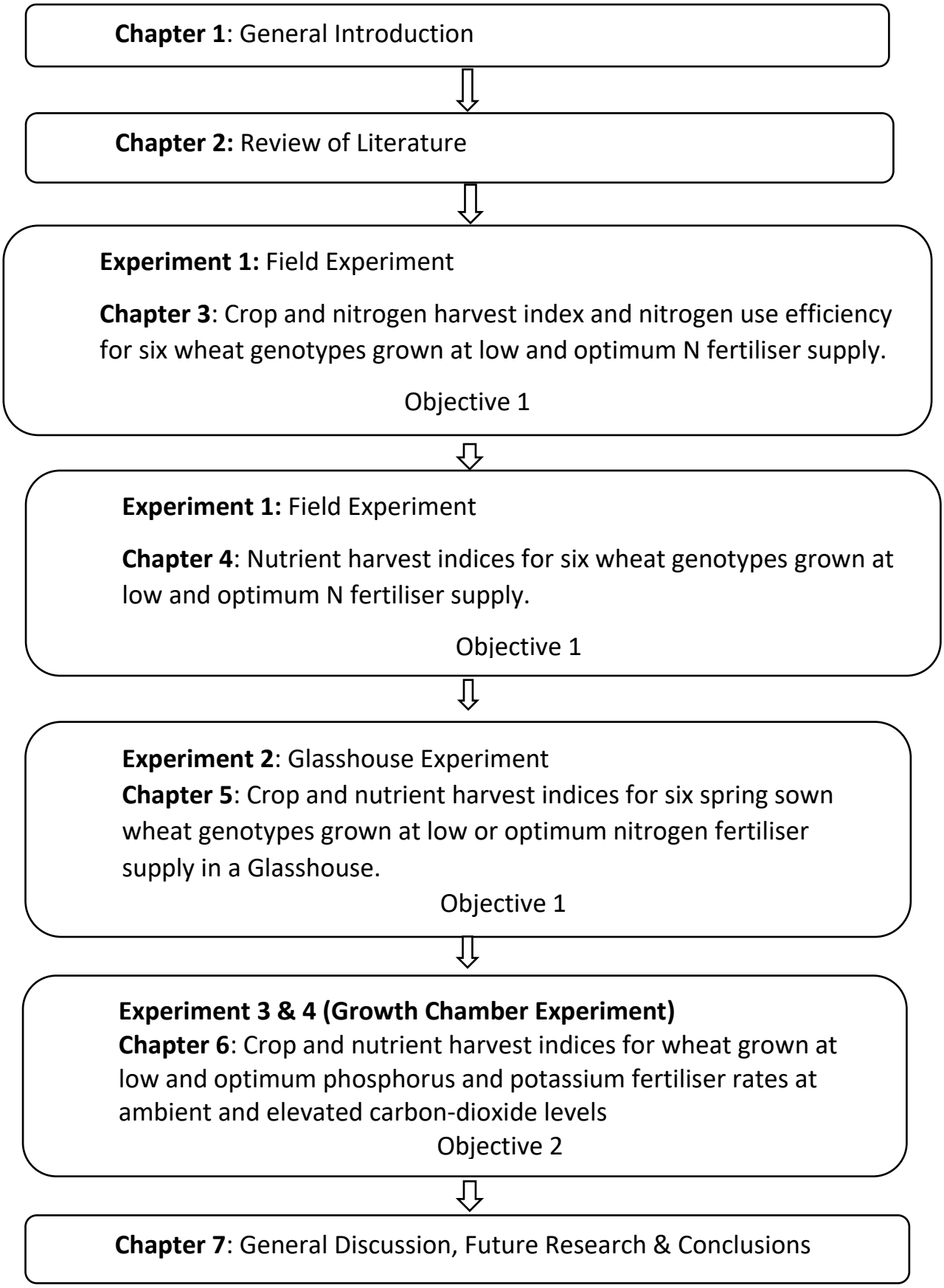

Figure 1.2: Outline of thesis structure

Chapter 2: Review of the Literature

2.1. Introduction

Inorganic fertiliser use has alleviated soil mineral nutrient (e.g. N) limitations in crops. It has also resulted in substantial increases in crop yield and soil fertility over the past century (Tilman et al. 2002). However, synthetic fertiliser applications (e.g. N and P) have dramatically altered global nutrient budgets, water quality, greenhouse gas balance, and their feedback to the climate system (Lu & Tian 2017).

The nutrient focus in high-yielding wheat production systems is often on N and occasionally P (Calderini et al. 1995a; Zhou et al. 2013). However it is also important to understand the temporal uptake and accumulation of other essential macro- [e.g. K, sulphur (S), magnesium (Mg) and calcium (Ca) and micro-nutrients (e.g. iron (Fe), manganese (Mn), Zinc (Zn), copper (Cu)). Balanced nutrition must be achieved to optimise wheat productivity (Hamnér et al. 2017). This may be gained by exploring the physiological link approach, i.e. nutrient uptake and partitioning at different crop growth stages. Nutrient accumulation studies with wheat have typically investigated macro-nutrients (Gregory et al. 1979; du Preez & Bennie 1991; Malhi et al. 2006). Studies including micro-nutrients are dated (Karlen & Whitney 1980; Miller et al. 1993; Hocking 1994) and involved older genotypes that may differ considerably from modern ones in terms of nutrient metabolism for both spring (Hocking 1994) and winter (LÁSztity et al. 1984) wheat. Modern crop production is characterised by high N use and high yielding genotypes. High N use can influence the concentration of other nutrients due to an over-proportional increase in biomass production in relation to nutrient uptake (Hamnér et al. 2017). This is particularly important because increased yield has been reported to affect food quality through the 'dilution' effect (Jarrell & Beverly 1981), leading to lower mineral content. This has been reported for several crops, but with inconsistent results (Davis 2009; Gooding et al. 2012), e.g. with declining Zn, Fe, Cu and Mg concentrations (Fan et al. 2008) or increasing Zn and Cu concentrations (Cakmak et al. 2010) with N fertilisation. This warrants further investigation to determine whether N fertiliser supply and the concomitant increase in biomass production in modern spring wheat genotypes influence the accumulation of other nutrients (Objective 1, Chapters 4 and 5).

The relative efficiency of assimilated carbon and N allocation to the harvested product can be represented by two key indices, the: (1) CHI (Donald 1962; Donald & Hamblin 1976) and (2) NHI (Austin et al. 1977; Austin et al. 1980; Löffler & Busch 1982; Dhugga & Waines 1989; Slafer et al. 1990). These indices measure the quantity and quality of the grain, respectively. A similar logic can be used for other macro- and micro-nutrients but this information is outdated (Miller et al. 1993; Hocking 1994) or only available for winter wheat (Weih et al. 2016; Hamnér et al. 2017). It is unclear how these values compare with spring sown, modern wheat genotypes. Therefore this study will determine temporal uptake patterns, accumulation and NuHIs for spring sown wheat genotypes grown at low or optimum N fertiliser supply (Objective 1, Chapters 4 and 5) or different CO_2, P and K levels (Objective 2, Chapters 6).

The improvement of CHI through the introduction of semi-dwarf genotypes (Austin et al. 1980), allowed the use of increased amounts of synthetic N fertiliser (Sections 1.1, 1.2). This review describes CHI as the key trait used to quantify above ground biomass (AGB) and grain yield. Equally, the proportion of nutrients remobilised to the grain (NHI, NuHI) is also described. From this, the quantity of grains produced per unit of available N or nutrient use efficiency (e.g. NUE) is quantified.

2.2 Crop harvest index (CHI)

Historical genetic gains in wheat yield since the 1960s have been associated with increased CHI (Donald 1962; Austin et al. 1980; Brancourt-Hulmel et al. 2003; Dai et al. 2016). CHI increases were attributed to increased grain yield and reduced straw in newer genotypes (Austin et al. 1980; Hay 1995). The mean CHI of wheat has increased from ~0.35 (pre—1955) to ~0.50 after the 1980s (Gifford et al. 1984; Austin et al. 1989; Berry et al. 2007). These gains were an indirect result of using semi-dwarf genes to reduce crop height to allow for greater use of synthetic N fertiliser (Donald & Hamblin 1976; Austin et al. 1980; Hay 1995; Austin 1999) as part of the 'green revolution' (Hedden 2003).

Currently, the maximum calculated CHI valuess of ~0.50 (Austin 1999; Berry et al. 2007; Senapati & Semenov 2019) have been reported for lodging-resistant winter wheat crops. These values are consistent with those reported in other parts of the world, for winter and spring wheat crops (Dai et al. 2016). These CHI values are less than, but approaching the

theoretical upper limit for wheat of ~0.62 suggested by Austin et al. (1980). This upper limit means further yield increases must also consider genetic enhancements that increase total biomass production (Calderini et al. 1995b; Austin 1999; Berry et al. 2007), while utilizing resources as efficiently as possible. This has been achieved in maize (*Zea mays* L), for which increases in grain yield potential in North America were largely attributed to increased total biomass yield (Russell 1991). This was achieved through breeding for more erect leaves (Duvick et al. 2004; Tollenaar & Lee 2006), which led to higher plant populations. Consequentially, earlier canopy closure and higher maximum green leaf area index (GLAI; the total one-sided green area of leaf tissue per unit ground surface area (Bréda 2003)) sustained greater leaf photosynthesis over the grain-filling period (Stone et al. 1998; Tollenaar & Dwyer 1999), which resulted in high grain yields. For wheat, the challenge is to identify crop characteristics that can increase resource use efficiency to underpin further yield improvements (Objectives 1 and 2, Chapters 3, 5 and 6), thus closing the gap between the current CHI and the theoretical upper limit of 0.62.

In general, higher and regular CHI values have been reported for temperate small-grain cereals (Figure 2.1) (Austin et al. 1989; Hay 1995), compared with less favourable environments, e.g. in Canada or Australia (Hucl & Baker 1987; Perry & D'Antuono 1989). These appear to have reached a plateau (Austin et al. 1980). The low CHIs for the less favourable regions have been associated with short grain filling periods due to water stress (*Loc. cit*), thus low thousand grain weights (TGW, g). This means representative CHIs for individual crop species, should be determined from crops grown under optimum growing conditions, and comparisons of CHIs should consider climatic region, especially for rain-fed crops. However, it should also be noted that, despite strong relationships between CHI and yield parameters (Dai et al. 2016), a high CHI does not necessarily suggest high grain yield or low straw yield, as assessments should be based on relatively constant biomass to be meaningful for yield comparisons among genotypes.

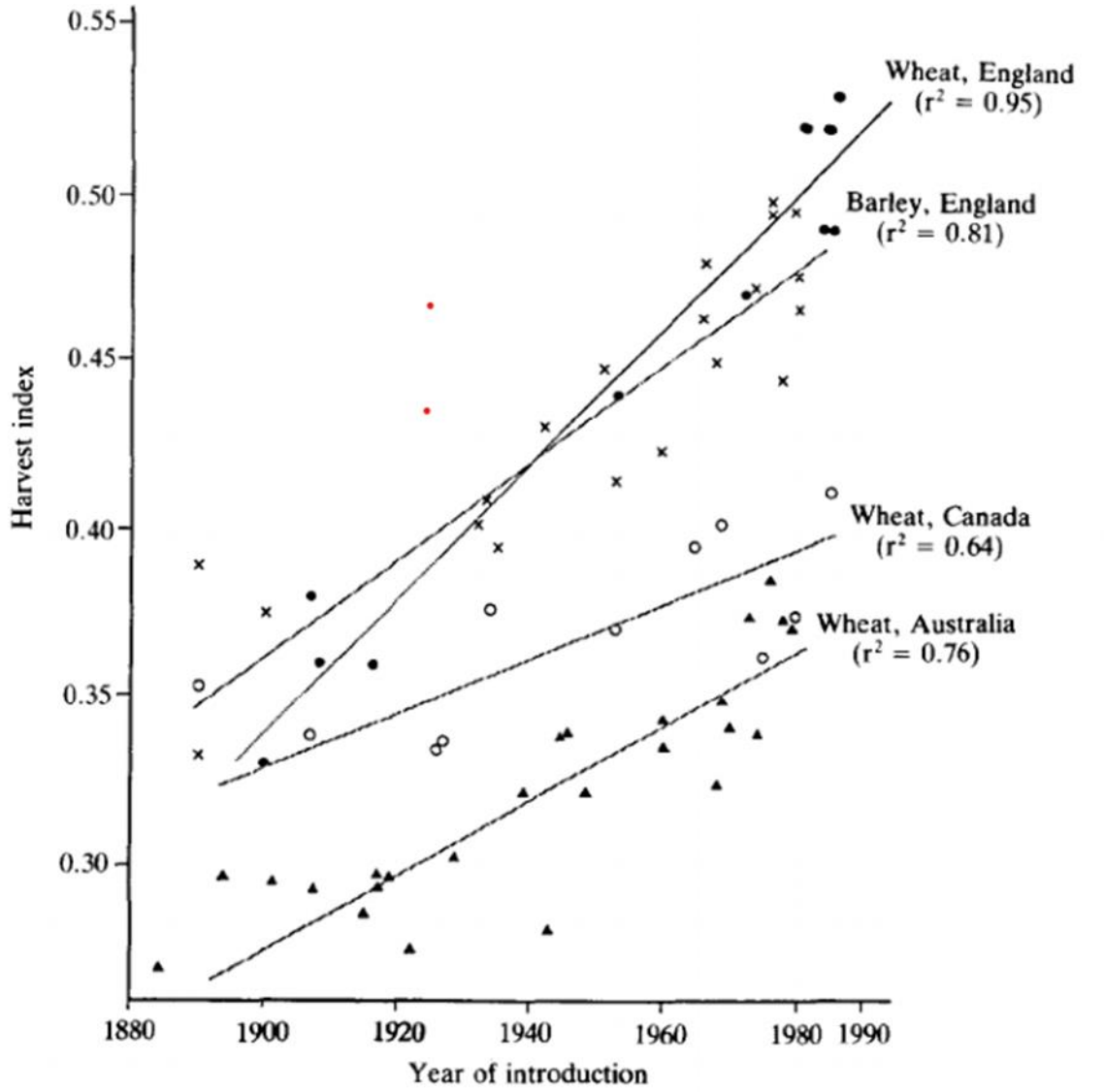

Figure 2.1: Relationships between crop harvest index and date of introduction of wheat varieties in England (●), Canada (○) and Australia (▲) and of barley varieties in England (X). Data from field experiments in which all varieties were grown under the same conditions (Austin et al. 1980; Riggst et al. 1981; Hucl & Baker 1987; Perry & D'Antuono 1989). The slopes of the regression lines indicate that the time required for crop harvest index to increase by 0.1 was 51 years (wheat, England), 74 years (barley, England), 160 years (wheat, Canada) or 97 years (wheat, Australia). Reproduced with permission from John Wiley & Sons, Inc, 2020.

CHIs have also been reported to increase with date of genotype introduction (Figure 2.1), and are higher for the semi-dwarf genotypes (bred post—1960s) as illustrated in a previous comprehensive review (Hay 1995). Progressive increases in CHI with date of introduction are an expression of competitiveness of the ears against the stem in semi-dwarf genotypes, which results in greater survival of florets per spikelets, thus a higher number of grains

(grain density) (Brooking & Kirby 1981). Austin et al., (1989) who reported 30% more grains per ear for the semi-dwarf compared with the older, taller genotypes confirmed this. Therefore, the higher CHI for the modern genotypes is a result of higher grain densities, coupled with relatively stable TGW (Bremner & Davidson 1978). Recent reports that suggest a trade-off between TGW and grain density are not purely speculative (Hawkesford 2014; Quintero et al. 2018), and were reported for wheat cultivars released in Australia between 1958 and 2007 (Sadras & Lawson 2011). In some genotypes, TGW is the key determinant of CHI, and the current study will determine the relationships between the yield components and their contribution to grain yield or CHI (Objectives 1 and 2, Chapters 3, 5 and 6).

The physiological basis of trends in the CHIs for cereal crops (e.g. wheat) has been attributed to the progressive increases in stem strength/ stiffness and decrease in stem length (Berry et al. 2007; Piñera-Chavez et al. 2016). This was achieved through the incorporation of dwarfing genes, such as the reduced height (*Rht*) gene (Jobson et al. 2019) from the Japanese Norin 10 wheat (e.g. Austin et al, 1980). Most of the historical studies (e.g. Austin et al., 1980, 1989, 1993; Perry and D'Antuono, 1989) have shown that increases in grain yield were mostly accounted for by the CHI. However, there are other reports that showed that grain yield increases were due to increased total biomass yield (e.g. Perry and D'Antuono, 1989 and Hucl and Baker, 1987). This gives confidence that increasing total biomass yield for modern genotypes is possible. Identification of physiological traits that can increase resource use efficiency to underpin further yield improvements in wheat is therefore important for future breeding selection (Objectives 1 and 2, Chapters 3, 5 and 6).

Previous reports show that CHIs differ with N supply (Hay & Walker 1989; Engel et al. 2003). Donald and Hamblin, (1976) reported that N fertiliser application resulted in increased , combined with a decrease in CHI, as the proportion of increases in exceeds that of the grain yield (McNeal et al. 1971). Furthermore, grain yield for modern wheat genotypes in the UK has been reported to be unresponsive to rates of N fertiliser application beyond 125—150 kg/ha, whereas biomass continued to increase beyond these rates. Nitrogen fertiliser applications within the optimum range commonly used have little effect on CHI (Ellen & Spiertz 1980), while super-optimal N fertiliser supply tends to cause reductions in CHI (Donald & Hamblin 1976; Austin et al. 1993). The focus in the current study the effects

fertiliser (N, P or K) on CHI for modern spring wheat genotypes grown under different environments (Objectives 1 and 2, Chapters 3, 5 and 6).

Little is known about the CHIs for modern spring sown wheat genotypes grown in New Zealand except for the historical report of 40 years ago (McEwan & Cross 1978) or those developed *in silico* (Senapati & Semenov 2019). Therefore the 'model' characteristics ('crop ideotype' (Donald 1968; Senapati & Semenov 2019)) known to influence photosynthesis, growth and grain production are less established for these modern spring sown wheat genotypes. Implicit is the assumption that all current breeding is based on the classical breeding approaches, of 'defect/ default elimination' or 'selection for yield', whose limitations have been elucidated (Donald & Hamblin 1976). As the limit to the theoretical upper limit for CHI is approached (Austin et al. 1980), genetic gain in yield will depend on detecting and exploiting genetic variation in biomass production, and alternative methods of breeding. To overcome the limitations of the classical breeding approaches, Donald (1968) proposed the 'breeding of crop ideotype' approach, which included breeding for model characteristics such as short and strong stem; few, small, erect leaves; low tillering capacity (oligoculm) and disease resistance. Breeding for such an ideotype has resulted in improved lodging resistance and higher CHI (Hamblin 1993; Berry et al. 2007). Even if scarce in the scientific literature, some ideotype breeding experiences have been successful (Gauffreteau 2018), resulting in an 8—15% increase in rice (*Oryza sativa* L) grain yield (Peng et al. 2008). The question remains to what extent these features have been utilised in modern wheat breeding programs, particularly under high yielding conditions in temperate maritime climates like New Zealand. Historical studies in New Zealand (McEwan & Cross 1978) indicated that semi-dwarf genotypes tended to be shorter and earlier flowering than their ancestors. Attempts to further increase the grain yield of early spring sown wheats in New Zealand, involved combining the high CHI of the semi-dwarf wheats with the high potential for AGB of some of the older cultivars (McEwan & Cross 1978) (Objectives 1 and 2, Chapters 3, 5 and 6).

2.2.1 Biomass accumulation and grain yield

The rate of biomass production by crops in non-stressed environments is directly related to the amount of intercepted incident photosynthetically active radiation (IPARi) (Biscoe & Gallagher 1977; Kiniry et al. 1989) and temperature (Section 1.3). Physiologically, increased

light interception occurs from canopy characteristics that extend the duration and efficiency of light capture and then utilise the additional biomass through a high CHI to maximize grain yields (Donald 1968; Semenov & Stratonovitch 2013). Conceptually, grain yield is the product of the amount of IPARi by canopies (Biscoe & Gallagher 1977), and the efficiency with which it is converted into biomass (RUE) (Monteith 1977; Gallagher & Biscoe 1978a). The CHI then quantifies the proportion of economically valuable biomass allocated to grains, as shown in Equation 2.1 (Gallagher & Biscoe 1978a; Araus et al. 2002):

$$\text{Grain yield} = PAR_{acc} \; x \; \text{RUE} \; x \; \text{CHI} \qquad \text{Equation 2.1}$$

Where PAR_{acc} is daily IPARi values accumulated throughout the growing season.

Despite the universal acceptance of these parameters to explain crop yields, there is little current research to examine the genetic variability for modern, spring wheat genotypes, and their interaction with N supply (Objective 1, Chapter 3 and 5). Genotypes that can attain high yields under low N supply are desirable and a current focus of plant breeding programmes (Lammerts van Bueren *et al.* 2011; Lammerts van Bueren and Struik 2017).

Further to the effects of IPARi on AGB and grain yield, the SLN (g N/m^2) has been show to affect photosynthetic capacity and therefore RUE (Sinclair & Horie 1989; Meinke et al. 1997) for a range of crops. For wheat, Meinke et al. (1998) defined a SLN threshold value of 1.1 g N/m^2 leaf (Section 1.2), below which RUE decreases. However, canopy leaf N (Dreccer 2006) as well as specific leaf area (Ratjen & Kage 2016) are influenced by the light gradient within the canopy. Thus, for determination of SLN thresholds, mutual shading within the canopy should be taken into account. The SLN for different spring wheat genotypes will be determined in Experiment 1 and 2 (Objective 2: Chapters 3 and 5)

2. 3 Nutrient harvest index

The concept of CHI has been extended to partitioning of nutrients (Section 1.1). Briefly, NHI for wheat has been extensively reported in previous studies. However, detailed knowledge about other NuHIs for modern spring wheats and how crop N concentration influences elemental translocation is currently lacking (Hamnér et al. 2017) (Objectives 1, Chapters 4 and 5). Available information is dated (Miller et al. 1993; Hocking 1994) or for winter wheat (Weih et al. 2016; Hamnér et al. 2017). In contrast, NuHIs have been reported for other crops, e.g. maize (Bender et al. 2013; Ciampitti et al. 2013; Ciampitti & Vyn 2013), soybean

(*Glycine max*. L. Merr.) (Bender et al. 2015; Bruns 2016) and rice (Fageria et al. 2008; Fageria & Oliveira 2014). Nutrient accumulation in maize (*Loc. cit*), suggests that most nutrients are accumulated before flowering, but some nutrients, such as P and micro-nutrients are often accumulated at later growth stages. The temporal pattern of nutrient uptake in modern spring wheat genotypes is unknown, and this study will determine these (Objectives 1 and 2, Chapters 4, 5 and 6), which may offer opportunities for differential nutrient application in future.

2.3.1 Nitrogen harvest index (NHI)

The NHI is an important index (Sections 1.1; 2.1) to measure translocation efficiency of absorbed N from vegetative plant parts to the grain (Gorjanović et al. 2011; Fageria 2014). The NHI is calculated as shown in Equation 2.2 (Foulkes et al. 2009b):

$$\text{NHI} = \frac{\text{Grain N (kg/ha)}}{\text{Above ground N (AGN; straw N+grain N;) at harvest maturity (kg/ha))}} \qquad \text{Equation 2.2}$$

Where above ground N (AGN; kg/ha) is the total amount of N in the aboveground plant (straw + grain) at harvest maturity. Equation 2.2 can be adapted for any other nutrient (Section 2.3.3), by replacing N with the relevant nutrient.

There is limited recent information on NHI for modern spring sown wheat genotypes except for the reported range of 0.79—0.84 for crops grown in the field and lysimeter (Noulas et al. 2004) and growth chamber (Andersson & Johansson 2006) experiments. These values are within the ranges reported for winter wheats of 0.70—0.90 (Slafer et al. 1990; Angus & Fischer 1991; Takahashi & Anwar 2007; Barraclough et al. 2010; Barraclough et al. 2014). However, these values are higher than those reported in historical studies of 0.64—0.75, for spring wheats (McNeal et al. 1966; McNeal et al. 1968; Desai & Bhatia 1978). The current study will confirm the values reported for spring wheats (Noulas et al. 2004; Andersson & Johansson 2006) and how they compare with the established values for winter wheat (Objectives 1 and 2, Chapters 3, 5 and 6).

While increasing NHI is possible in theory (Barraclough et al. 2010), in practice the physiological processes leading to increased yield or grain N concentration (N_g%) are considered mutually exclusive (Sinclair & de Wit 1975). This is because the duration of the grain development period is intimately tied to the rate of N uptake during grain-fill. A low

rate of N uptake results in a large N demand from the grains and leads to extensive N translocation from vegetative tissue. The consequence is premature senescence and a shorter period of grain development, and lower total yield. For cereal crops, grain growth may be sustained by a relatively small rate of uptake of N during the grain-filling period (post-anthesis N uptake; PANU (Equation 3.4)) without the necessity of N redistribution within the plant (*Loc. cit*). However, as the amount of N available from the soil is often finite, these crops will have to translocate N during grain development to support yield formation. The implication is that N fertilisation is important to partially alleviate the limitation of total soil N availability, thus, increased yield and NHI, by postponing leaf senescence. This study will confirm the effects of N stress in leaf senescence on the grain-filling period, yield and NHI for spring sown wheat (Objective 1, Chapters 3 and 5).

Previous reports show inconsistent NHI response to N supply. The NHI declined with increased N fertiliser supply (Halloran 1981; Klikocka et al. 2017; Belete et al. 2018) for spring wheat and was unaffected (Barraclough et al. 2010; Barraclough et al. 2014) for winter wheat. In other cereal species, such as oats (*Avena sativa* L.), NHI decreased with increased N supply (Rattunde & Frey 1986). There is a positive relationship between CHI and grain yield for winter wheat (Austin et al. 1977; Fageria 2014), which implies that more N has to be translocated to the grain to maintain quality. A positive relationship between NHI and grain yield has been reported in other grain crops, such as oats (Rattunde & Frey 1986). Furthermore, positive relationships between NHI and CHI for spring-sown wheats (Desai & Bhatia 1978), indicated that the distribution of N between straw and grain to a large extent, but not entirely, depends on the partitioning of dry matter between the two components. In spring sown wheat, the proportion of N at anthesis can be as high as 90—100% of the total N in the plant at maturity (Campbell et al. 1977; Löffler & Busch 1982), resulting in low yield and NHIs (Dhugga & Waines 1989). In winter wheat, some genotypes continued taking up appreciable quantities of N after anthesis (during grain filling) (Austin et al. 1977), which results in higher yield and NHIs. However, PANU delays leaf senescence, allowing a longer grain filling period and hence a higher grain yield (Zhao et al. 2015), but delays N translocation to the grain. Therefore, to maintain yield and NHI, there has to be high levels of available soil N from either soil mineralisation or synthetic N fertiliser application, and more efficient N translocation.

Previous reports have shown that NHI differed among wheat genotypes for both winter and spring wheats (Austin et al. 1977; Desai & Bhatia 1978; Dhugga & Waines 1989; Gorjanović et al. 2011). Similar results have been reported for oats (Rattunde & Frey 1986). The high NHI was associated with efficient utilisation of N (Fageria & Baligar 2005). The variations in NHI are characteristics of genotypes that may be useful in selecting crops for future breeding, resulting in higher grain yield and quality (Fawcett & Frey 1982) or increased yield, with constant N content (Löffler & Busch 1982). Timing of N uptake in wheat is important for both grain yield and NHIs (Dhugga & Waines 1989), with genotypes that take up most of the N pre-anthesis reported to produce low yield and NHIs, as the increased N demand from the developing grains will result in pre-mature leaf senescence. In this study, nutrient uptake will be determined at anthesis and harvest maturity growth stages to establish the patterns of nutrient accumulation (Section 2.5.1.1) (Objectives 1 and 2, Chapters 3, 5 and 6).

2.3.2. Nitrogen use efficiency (NUE)

Nitrogen use efficiency (NUE; Equation 2.3) is the grain dry matter (DM) per unit N available, from applied fertiliser and/or soil stocks (Section 1.2) (Moll *et al.*, 1982).

$$\mathrm{NUE} = \frac{\text{Grain DM yield (kg/ha)}}{\text{Amount of N supplied (N; kg/ha)}} \qquad \text{Equation 2.3}$$

Where the amount of N supplied to the plant is from the soil and/or fertiliser applied.

The NUE is subdivided into components that identify soil (N uptake efficiency; NupE) and plant (N utilisation efficiency; NutE) processes that contribute to the overall use of N, as summarised in Equations 2.4 and 2.5 (Moll et al. 1982; Le Gouis et al. 2000). The NupE is the ratio of the N recovered in aboveground biomass (AGN) to the total N supplied to the crop:

$$\mathrm{NupE} = \frac{\text{Above-ground N (AGN; kg/ha)}}{\text{Amount of N supplied (N; kg/ha)}} \qquad \text{Equation 2.4}$$

The NutE is the ratio of grain yield to the total AGN in the crop:

$$\mathrm{NutE} = \frac{\text{Grain DM yield (kg/ha)}}{\text{AGN (kg/ha)}} \qquad \text{Equation 2.5}$$

The apparent N fertiliser recovery (AFR) was calculated by the 'difference' or 'N balance' method and determined from these data as shown in Equation 2.6 (Foulkes et al. 1998):

$$\mathrm{AFRopt} = \frac{(\text{AGN N opt} - \text{AGN N zero})}{\text{N opt}} * 100 \qquad \text{Equation 2.6}$$

Where 'AGN N opt and AGN N zero' are the crop N yields of the optimally fertilised (N opt) and unfertilised (0 kg N/ha) crops.

It is estimated that the average AFR for winter wheat is 30-50% (Raun et al., 2002), although higher rates (>60%) have been reported (Sylvester-Bradley & Kindred 2009). The high AFR rates have led to increased global emphasis in breeding wheat cultivars with improved NUE (Hirel et al. 2007), thus leading to reduced fertiliser N inputs while maintaining grain yields. However, little is known about the N recovery for modern spring sown wheat genotypes, and this study will determine both NUE and AFR for spring wheat genotypes (Objectives 1, Chapter 3 and 5).

2.3.3 Nutrient harvest index (NuHI)

The paucity of data regarding NuHIs, except NHI (Section 2.3.1) for modern spring wheat production systems necessitates an understanding of nutrient uptake and partitioning (Objectives 1 and 2, Chapters 4, 5 and 6). Furthermore, the influence of N concentration on nutrient partitioning for spring wheat is also lacking. This warrants further investigation to determine how N fertiliser supply and the concomitant increase in spring wheat AGB production influences the NuHIs (Objectives 1 and 2, Chapters 4, 5 and 6). Studies on NuHIs for both spring and winter wheats have concentrated on N (NHI, Section 2.3.1), and P harvest index (PHI) (Clarke et al. 1990; Batten 1992; Rose et al. 2007; Takahashi & Anwar 2007; Rose et al. 2013; McDonald et al. 2015) and sparingly on S harvest index (SHI) (Klikocka et al. 2017) and K harvest index (KHI) (Rose et al. 2007). However, NuHIs for the other macro- (Mg or Ca) and micro-nutrients for modern spring wheat genotypes have not been investigated, with the only recent, comprehensive study reported for winter wheat (Hamnér et al. 2017), which warrants further studies in this area (Objectives 1 and 2, Chapters 4, 5 and 6).

The PHIs of modern wheat genotypes range of 0.80—0.90 for spring (Clarke et al. 1990) and winter (Takahashi & Anwar 2007) wheats, are at the upper end of the NHI (Section 2.3.1). These high PHI values are attributed to the short stature of the semi-dwarf wheats (Jones et al. 1989), similar to the conclusions drawn for NHI (Austin et al. 1980). In a comparison of standard (old, tall) and semi-dwarf genotypes, Jones et al. (1989) reported that P efficient genotypes needed a low PHI, i.e. the ability to retain P in the straw at senescence so that P

export from the farm system is minimised. McDonald et al. (2015) reported a strong relationship between PHI and CHI, indicating that partitioning of P was strongly related to the partitioning of biomass. These data confirmed the numerous reports in the literature (e.g. Dhugga and Waines 1989) that dry matter accumulation patterns, rather than nutrient (e.g. N) concentration differences, determine total nutrient uptake. Furthermore, Clark et al. (1990) reported greater PHI than NHI regardless of the treatment and attributed this to the higher translocation efficiency for P than N.

The SHI values for wheat are inconsistent, higher for winter wheat at 0.45-0.55 (Monaghan et al. 1999; Hamnér et al. 2017), compared with ≤0.35 reported for spring wheat (Manderscheid et al. 1995). In other crops, e.g. maize, SHI values of 0.60 have been reported (Ciampitti et al. 2013). Similarly, the KHI values are also lower for spring wheat at ≤0.20 (Hocking 1994; Manderscheid et al. 1995), compared with ∼0.30 for winter wheat (Hamnér et al. 2017). Lower KHI values have been reported for other crops, such as maize (0.28) (Ciampitti et al. 2013). As there is no SHI or KHI reported for modern spring wheat genotypes, and how N fertiliser supply will influence these indices, the current study will determine these NuHIs (Objectives 1 and 2, Chapters 4, 5, and 6).

The limited information on CaHI and MgHI, shows values of 0.11—0.13 and 0.30–0.60, respectively, which are dated for spring wheat (Hocking 1994; Manderscheid et al. 1995) or for winter wheat (Hamnér et al. 2017). These values are consistent with those reported for maize (Ciampitti & Vyn 2013) of 0.05 (CaHI) and 0.44 (MgHI). Reported NuHIs for micro-nutrients were inconsistent, higher for winter wheat at 0.63 (Fe), 0.80 (Zn), 0.57 (Mn) and 0.75 (Cu) (Hamnér et al. 2017), compared with 0.05 (Fe), 0.67 (Zn), 0.34 (Mn) and 0.36 (Cu) reported for spring wheat (Hocking 1994). These NuHIs are from a single genotype, grown under optimum nutrient supply (Hocking 1994), and therefore the effects of N fertiliser supply on micro-NuHIs, on a number of spring sown modern wheat genotypes will be determined in the current study (Objectives 1 and 2, Chapters 4, 5, and 6). In maize, the respective micro-NuHIs were lower than reported for wheat, at 0.17, 0.52, 0.14 and 0.32, respectively. Overall, the NuHIs ranged from ≤ 0.20 for Ca, Fe and K to moderate values of 0.30—0.60 for Cu, Mn, Mg, S and high values of ≥0.60 for N, P and Zn (Hocking 1994).

2.3.3.1 Evidence for genetic variation in N-use efficiency

Relative contributions of the component NUE traits (NupE and NutE) to genetic variation in NUE has been inconsistent (Foulkes et al. 2009b). Under low soil N conditions, genetic gains in NUE have been related to improvements in both NupE (Van Sanford & MacKown 1986; Dhugga & Waines 1989) and NutE (Fischer & Wall 1976; Brancourt-Hulmel et al. 2003). However, under high N supply, most of the reports show that wheat breeding consistently resulted in improved NutE, associated with higher CHI (Brancourt-Hulmel et al., 2003). Genetic variation in NUE has also been demonstrated for maize (Moll & Kamprath 1977; Moll et al. 1982) and barley (Anbessa et al. 2009). Overall conclusion from these results was that when N was limiting the ability to explore the soil and absorb N, NupE is of greater importance to the crop. Conversely, when N is not limiting, sufficient N will be available within the crop independent of the efficiency of the root system, and NutE would be of greater importance in determining NUE. There are limited reports on the use of physiological traits (Ortiz-Monasterio et al. 1997) for selection in breeding to improve wheat NUE.

2.3.3.2. Rationale for improved NUE

Nitrogen fertilisers have a significant production cost in dollars and greenhouse gas emissions. They also have negative environmental impacts (Foulkes et al. 2009b; Pask et al. 2012), associated with nitrate leaching from excessive use, leading to eutrophication of rivers and lakes, and global warming due to emissions of nitrous oxides (Sylvester-Bradley and Kindred, 2009; Gaju et al., 2011). Conversely, at global level, wheat is the most widely grown crop (Section 1.1) and therefore there is a need for improved breeding of wheat cultivars (Hirel et al., 2007; Foulkes et al., 2009) to manage both fertiliser costs and minimize negative environmental consequences. Key to achievement of these objectives is the identification of physiological traits that are easily transferrable into new cultivars through breeding (Objectives 1 and 2, Chapters 3, 5 and 6). This coupled with improved N management strategies (Cassman et al. 2002; Shanahan et al. 2008) can lead to potentially increased NUE, increased grain yield per unit of N supplied. In the current study, canopy traits important in total biomass production were investigated (Objectives 1 and 2, Chapters 3, 5 and 6).

2.4 Crop nutritional requirements

The nutritional requirements of crops have always been of interest, more so now than before with changing economic and environmental dynamics in crop production (Bruns 2016). The general crop nutrient requirement is illustrated in Figure 2.2 (Brady & Weil 2017). For each nutrient there is a sufficiency rate, below which crop production is limited and above which there is no yield response and it becomes costly both financially and environmentally. Excess nutrient supply can also lead to toxicity, thus reduced growth.

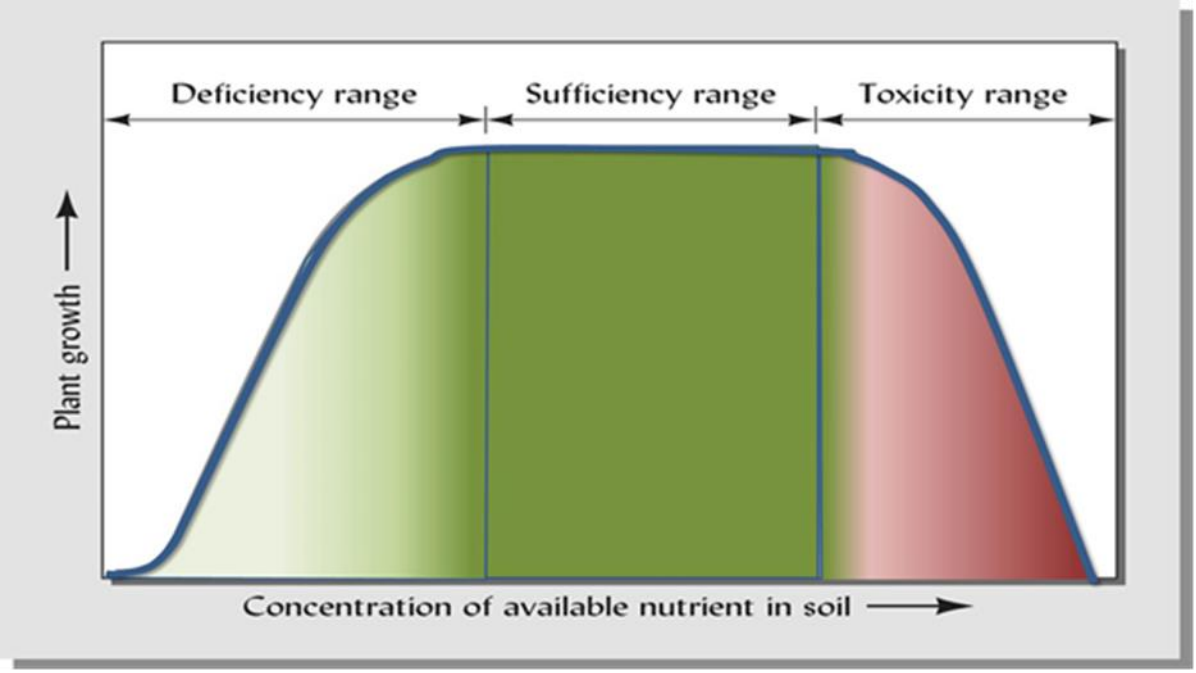

Figure 2.2 Relationship between plant growth and health and amount of nutrient available (Brady and Weil, 2017). Reproduced with permission from Brady and Weil, (2017). List of plant nutrients is in Figure 2.3 and critical concentrations are in Table 2.1.

The identification of the period of maximum nutrient demand and knowledge of total nutrient uptake is required to develop best nutrient management practices (du Preez & Bennie 1991; Miller et al. 1993). This knowledge also provides data to enhance accuracy of simulation models for predicting wheat growth. At the farm level, knowledge of nutrient uptake patterns allows tactical decisions on optimum times to apply nutrients based on crop demand and soil supply. Nutrient uptake patterns and accumulation will be determined in the study for spring sown wheat crops grown in different environments (Objectives 1 and 2, Chapters 4, 5 and 6).

Mineral nutrients are divided into two groups (McLaren & Cameron 1996; Mengel et al. 2001; Wakeel et al. 2011) as shown in Figure 2.3: macro-nutrients (e.g. K and Mg) and micro-nutrients (e.g. Fe and Zn). Macro-nutrients are needed or found in plants in relatively higher amounts than micro-nutrients. Plant tissue concentration of macro-nutrients could be a thousand times greater than the concentrations of micro-nutrients and are generally expressed as percentages of dry matter (%DM) or g/kg DM, whereas micro-nutrients are expressed as mg/kg DM. In this study all macro-nutrients and selected micro-nutrients' accumulation and partitioning will be considered in relation to the imposed treatments (genotypes, N fertiliser supply, CO_2 level, P and K fertiliser supply) (Objectives 1 and 2, Chapters 3— 6).

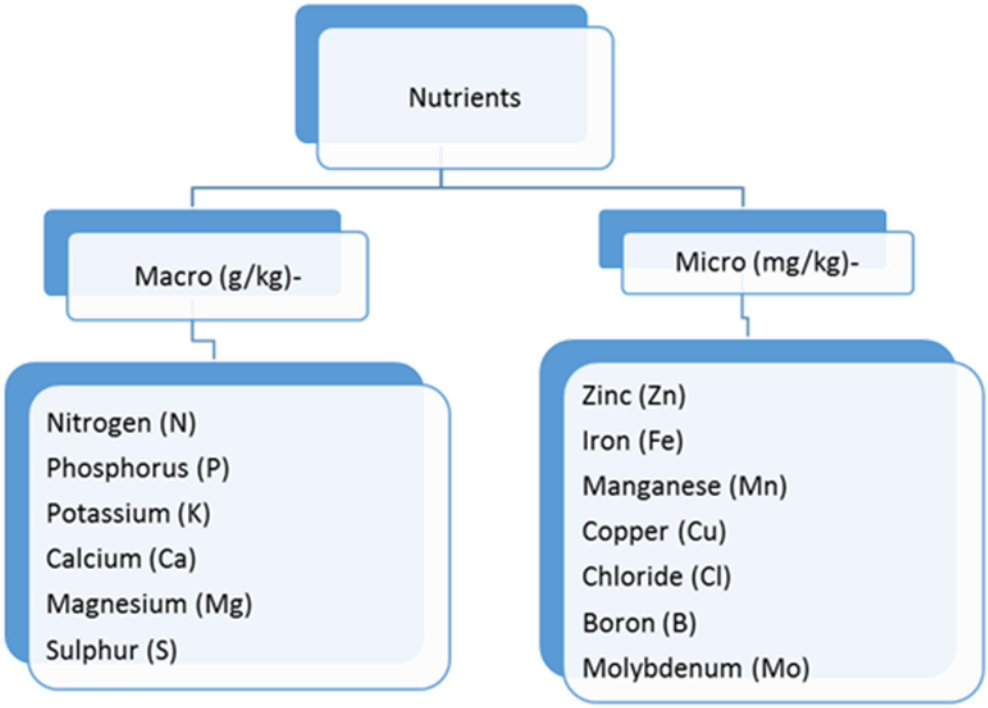

Figure 2.3: Essential nutrients required by higher plants. Source: Drawn by author based on published data (McLaren & Cameron 1996; Mengel et al. 2001). Full details on functions, typical concentration (%DM or mg/kg DM), deficiency symptoms and mode of transport have been summarised in Appendix 2.1.

The essential nutrients (Figure 2.3) required by higher plants are exclusively inorganic (McLaren & Cameron 1996; Mengel et al. 2001). These are defined based on three criteria (Arnon & Stout 1939): 1. a deficiency of it makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle; (2) such deficiency is specific to the element

in question, and can be prevented or corrected only by supplying this element; and (3) the element is directly involved in the nutrition of the plant quite apart from its possible effects in correcting some unfavourable microbiological or chemical condition of the soil or other culture medium. The functions of the macro- and micro-nutrients have been comprehensively discussed in several reviews (McLaren & Cameron 1996; Mengel et al. 2001), and are summarised in Appendix 2.1.

2.5. Nutrient uptake and partitioning

2.5.1. Whole crop

Plant nutrients are moved between sites of uptake and production and sites of consumption through the vascular tissues of the xylem and phloem (Mengel et al. 2001) as summarised in Appendix 2.1. Nutrients are taken up from the root medium and translocated towards the upper plant parts through the xylem. Nutrient movement in the xylem is one directional, upwards, while movement in the phloem is bidirectional. Other nutrients are translocated through the xylem and phloem. Plant nutrients can be grouped into three categories based on their mobility in the phloem (Loneragan et al. 1976; Miller et al. 1993; Hocking 1994; Reuter & Robinson 1997): mobile nutrients (e.g. N, P, Mg and K), immobile nutrients (e.g. Ca, Fe and Mn) and nutrients of restricted mobility (e.g. S, Cu and Zn).

Knowledge of the nutrient uptake patterns in wheat grown under field conditions is useful for understanding nutritional problems experienced by the crops (Karlen & Whitney 1980). These authors showed that the general trend in nutrient concentration dynamics followed the timing of assessment (autumn v. spring). For spring sown wheat (e.g. Hocking 1994), the concentrations for phloem mobile nutrients, e.g. N and P (Reuter & Robinson 1997) have been reported to be highest in the shoot early in the season and to decrease with plant age. This was attributed to the relatively slower nutrient uptake than carbon assimilation (Gregory et al. 1979). However, the concentration of phloem immobile nutrients generally increased in vegetative organs throughout the season. Similar results were for winter wheat (Karlen & Whitney 1980). In light of these reported changes in nutrient concentration with crop age (Hocking 1994), it is important to identify the growth stages when sampling crops for nutrient analyses in relation to critical nutrient concentrations. Nutrient partitioning has been reported to differ with nutrient mobility (*Loc. cit*), with higher concentration of mobile

nutrients reported for the grains, and the immobile nutrients (e.g. Ca and Fe) in other plant organs. However, there are two exceptions to this rule (Miller et al. 1993): K (highly phloem mobile) and Mn (phloem immobile), which accumulate more in the straw and grain, respectively. This pattern of K distribution has been reported in other cereals, e.g. grain sorghum (Hocking 1993) and maize (Karlen et al. 1988; Ciampitti et al. 2013), and could be attributed to the fact that K is a constituent of both lignin and cellulose, which make up the stems. This nutrient accumulation behaviour will be investigated in this study (Objectives 1 and 2, Chapters 4, 5 and 6).

For winter wheat, N, P and K concentrations decreased slightly during autumn, but never fell below the critical levels (Appendix 2.1; Table 2.1). Specifically, the crop N concentration decreased linearly from 5 to 3.5% during autumn and further to ~1.0% as the plant approached physiological maturity in spring. Other nutrients (P, K, S, Mn and Cu) followed the same trends (Karlen & Whitney 1980), while Mg and Zn concentrations in the crops were constant throughout the growing period at < 0.20% and ~25 mg/kg DM, respectively. However, the Ca response was variable, decreased in autumn, stable at ~0.45% in spring until grain filling, and then decreased to ~0.25% as the plants matured. Most of the Ca is stored in the older leaves; hence, there is low translocation to the grain.

As these data were obtained from a single, winter wheat genotype ('Centurk') (Karlen & Whitney 1980), grown under adequate nutrient levels, there is a need to extend these investigations to include 'modern' spring wheat genotypes grown under different N fertiliser supply conditions. In the current study, temporal uptake patterns and accumulation of nutrients will be determined at anthesis and harvest maturity (Objectives 1 and 2, Chapters 4, 5 and 6). Furthermore, the effects of CO_2, P and K levels on temporal uptake and accumulation of nutrients will also be determined (Objective 2, Chapters 6).

Table 2.1: Critical composition[1] and optimum ranges for nutrients in wheat at booting stage, for diagnostic interpretations of total plant analyses.

Nutrient	Wheat	Optimum values
Nitrogen (%)	2.60*	2.50-4.00**
Phosphorus (%)	0.30*	0.25-0.50**
Potassium (%)	1.80*	1.50-4.50**
Calcium (%)	0.35*	0.25-1.00**
Magnesium (%)	0.15*	0.10-0.30**
Sulphur (%)	0.15***	0.10-0.40***
Manganese (mg/kg DM)	30.0*	10.0-50.0****
Iron (mg/kg DM)	25.0*	10.0-100*****
Zinc (mg/kg DM)	15.0*	10.0-25.0****
Copper (mg/kg DM)	5.00*	2.00-20.0****

[1] These values pertain to the whole plant at the boot stage (Melsted et al. 1969), and the optimum figures have been sourced from difference reports: *(Melsted et al. 1969), **(Bergmann & Bergmann 1985; Reuter & Robinson 1997), ***(Jones 1986), ****(McLaren & Cameron 1996) and *****(Mengel et al. 2001; Reuter & Robinson 1997).

2.5.1.1 Temporal pattern of nutrient accumulation

Previous studies on the pattern of nutrient uptake in wheat have been inconsistent (Miller et al. 1993; Hocking 1994; Hamnér et al. 2017). For the spring sown crops (Hocking 1994) the proportion of AGB at anthesis was 65.2% and nutrient uptake was 75—100% of Mg, Cu, S, P, N and K, and 55—70% of Mn, Fe, Zn and Ca. In contrast, for winter wheat, Hamnér et al., (2017) reported the proportion of AGB at anthesis of 74.4%, and the corresponding nutrient uptake followed AGB accumulation for P, Mg, Zn, Cu and Mn, while K, Ca, N, S and Fe accumulated faster and hence were mostly taken up pre-anthesis. These results show that uptake of nutrients in spring sown crops was faster than biomass accumulation for most of the macro-nutrients compared with the winter wheat. However, Miller et al., (1993) reported lower values, with ∼64%, and ∼45% of the total aerial N and P, respectively, accumulated by anthesis. Demand for these nutrients is high in early growth (*Loc. cit*), by early tillering for N and flag leaf stage for P. However, the bulk of the Ca (60%), Cu (50%) and Mg (70%) was accumulated post-anthesis. The low nutrient uptake early in the growing

season (e.g. Miller et al., 1993), could at least partly, be compensated for by late season uptake with possible implications for nutrient management. Furthermore, total nutrient amounts and concentrations of most nutrients (except P and Mn) increased with N uptake for winter wheat (Weih et al. 2016; Hamnér et al. 2017). Similar results have been shown for maize, where 91% of Ca, and 100% of K were taken up pre-anthesis (Ciampitti et al. 2013; Ciampitti & Vyn 2013), compared with 47—60% of P, S, Fe, and Zn, and about 73 % of Mg, Mn and Cu. Temporal nutrient uptake patterns for modern spring wheat genotypes in New Zealand have not been established, thus the implications for nutrient management are unknown. In this study, temporal uptake and accumulation of nutrients for spring wheat genotypes will be determined across different environments (Objectives 1 and 2, Chapters 4, 5 and 6).

The N: mineral nutrient ratios have been used as diagnostic tools for nutrient limitation (Sadras 2006). These relationships have been established for winter wheat (Hamnér et al. 2017) and maize (Ciampitti et al. 2013; Ciampitti & Vyn 2013), but not for spring-sown wheat crops. However, it has been shown that the bulk of the N and P accumulated in the vegetative organs is remobilised to the grains (Gregory et al. 1979), but it is still unclear for many other nutrients. In maize, results show that some nutrient concentrations increased at higher N rates (e.g. P, K and S) whereas other nutrients are negatively correlated to N concentration (*loc. cit*). Similar results have been reported for winter wheat (Hamnér et al. 2017). Nutrient uptake and partitioning are also affected by environmental factors that affect plant growth and development, such as solar radiation and temperature, respectively (Ritchie et al. 1998) and the balance between CO_2 and O_2 (Bloom 2006). The effects of N fertiliser supply on N: nutrient ratios will be determined in this study (Objectives 1, Chapters 4 and 5).

2.6 Elevated carbon-dioxide

Atmospheric concentration of CO_2 has risen by ~52% in the last century; from about 270 µmol/mol in pre–industrial times (Whorf & Keeling 1998) to ~410 µmol/mol at present (Zheng et al. 2018; NOAA 2020) and is estimated to reach 500—1000 µmol/mol by the end of the 21st century (Fung et al. 2005; IPCC 2014). This will lead to changes in the global climate (Long et al. 2004; Norby & Luo 2004), influencing plant life, and consequently future food production. Most of the research on the effects of plant nutrition on crop growth and

yield under eCO_2 has focused on N and sparingly on P (Section 1.3). Responses to K have not been considered under changing climatic conditions. Both P and K are essential element of the modern agricultural system, but finite resources (Dhillon et al. 2017; Dhillon et al. 2019) and therefore their long-term management is important. Knowledge of P and K uptake patterns and partitioning could help in determining timing and rate of application, which could result in improved P and K use efficiency. The effects of CO_2, P and K level on temporal nutrient uptake will also be investigated in this study (Objective 2, Chapter 6).

There is now a significant body of experimental data available on the effects of eCO_2 on wheat yield, as reported in comprehensive historical (Kimball 1983; Cure 1985; Cure & Acock 1986; Lawlord & Mitchell 1991) and recent (Amthor 2001; Broberg et al. 2019) reviews. Amthor, (2001) reported that doubling CO_2 concentration to 700 µmol/mol increased yield by ~31%. However, there is limited information on how other macro-nutrient (e.g. P and K) affect CHI, NHI, NuHI and hence the grain yield and quality under eCO_2. The effects of P and K fertiliser supply on CHI, NHI and NuHI will be determined in this study (Objective 2, Chapters 6).

Recent work has shown that most of the mineral nutrient concentrations decreased under eCO_2 (Loladze 2002; McGrath & Lobell 2013) and the reductions differed among crop species, and crop organ. The decline in nutrient concentration at eCO_2 can be explained two-fold (Conroy 1992; Taub et al. 2008; Taub & Wang 2008; McGrath & Lobell 2013): (1) dilution by enhanced carbohydrate production and (2) a reduction in nutrient uptake due to the CO_2 induced decrease in transpiration rate. This has been consistent for a number of cereal crops, such as wheat, barley and rice (Manderscheid et al. 1995; Kobayashi et al. 2006). Most of the work on nutrient concentrations has been done for N (McGrath & Lobell 2013), and the changes in other mineral nutrients are less well studied. Manderscheid et al. (1995) indicated that the response to eCO_2 differed with mineral nutrient, as well as the crops species, with a range of 2—20% decrease in concentration for nutrients such as Mg, Zn and Fe in cereals. However, Ca concentration has been found to increase in rice (Seneweera & Conroy 1997). Little is known about how mineral nutrient concentrations respond to P and K deficiency under eCO_2, for modern spring wheat, and therefore this study will be used to establish those values (Objective 2, Chapters 6).

2.7 Gaps in literature:

- As the CHI of modern genotypes has plateaued, knowledge of alternative approaches to future breeding selection through 'identified' physiological traits that increase AGB yield and its partitioning in wheat is lacking.
- There is paucity of data regarding NuHIs, except NHI, thus grain quality important for human health is poorly understood. Where values are available, they are dated or from single genotypes grown under optimum nutrient supply, and therefore the effects of fertiliser (N, P and K) and CO_2 supply on NuHIs of modern spring wheat genotypes needs further investigation.
- Little is known about temporal nutrient uptake (except for N) and the opportunities this may offer for differential nutrient application in future.

Chapter 3: Crop and nitrogen harvest index and nitrogen use efficiency for six wheat genotypes grown at low and optimum N fertiliser supply.

3.1 Introduction

Biomass and N accumulation and their distribution into the harvestable part of the crop (grain) are major drivers of yield (Lee & Tollenaar 2007). The value of wheat as a food crop can be represented by two key indices (Section 2.1): (1) CHI (e.g. Donald & Hamblin 1976) and (2) NHI (e.g. Austin et al. 1977; 1980). The two indices measure the quantity and quality of the grain, respectively.

The CHI reflects the partitioning of photosynthate between the grain and the vegetative components (Sinclair 1998) and improvements in CHI emphasize the importance of carbon allocation to grain production. The CHI for modern wheat genotypes (Section 2.2), is lower than the theoretical upper limit estimate of ~0.62 (Austin et al. 1980). Implicit is the assumption that genotypes with low CHIs, indicate that further improvement in partitioning of biomass would be possible. The CHI has been shown to differ among genotypes (Stapper & Fischer 1990; Dai et al. 2016), but results on effects of N fertiliser supply on CHI have been inconsistent (Hay 1995; Engel et al. 2003). Responses of CHI to genotypes and N fertiliser supply were described in Section 2.2.

Nitrogen is a critical component of the grain; therefore, there is a close relationship between NHI and CHI (Cox et al. 1986; Sinclair 1998). Genetic variability for NHI exists within crop genotypes and high NHI was associated with high NUE, e.g. in rice (Fageria & Baligar 2003). Thus, the variation in NHI among genotypes is a trait that may be useful in selecting future genotypes for higher grain yield.

The aim of this chapter is to establish CHI, NHI and NUE for six modern spring New Zealand wheat genotypes. This will be achieved through quantification of the agronomic response (biomass and grain yield), grain quality (N uptake and partitioning) and determination of canopy (e.g. leaf area size and expansion) and phenology (e.g. duration of grain filling) traits for the six wheat genotypes grown under low or optimum N fertiliser supply. The objective is to determine the effects of genotype, N fertiliser supply and their interaction on CHI, NHI

and NUE for spring wheat. The null hypothesis is that CHI, NHI and NUE of the six wheat genotypes will not be affected by the genotype, N fertiliser supply and their interaction. This chapter provides the underlying framework for subsequent experiments.

3.2 Material and Methods

This section provides details for Experiment 1 – a field experiment in 2017—2018 season at the Lincoln University cropping farm [43°37'37"S 172°28'2"E]), Lincoln, Canterbury, New Zealand. Measurements carried out during the seasons (Section 3.3) were grouped chronologically; hence, common sections have been used for their descriptions.

3.2.1 Experimental Site

The site is at an altitude of 10 m above sea level. At this location, spring wheat is typically sown in early- to mid-September and harvested in early February. The climate at Lincoln is temperate maritime, with mild to cool winters and warm summers (Figure 3.1; Section 3.4). The mean annual rainfall is ~600 mm, distributed evenly throughout the year and the average annual temperature is 12.0°C (23°C summer maximum and 1°C winter minimum) (NIWA 2019). The mean annual incident solar radiation is ~ 4930 MJ/m^2. Weather data from a nearby Broadfield meteorological weather station (43°37'34.4"S 172°28'13.4"E; Agent number: 17603), located within 200 m of the experimental site and average long-term climate, for a 30 year period, 1970-2010 (NIWA 2014) are described in Section 3.4.

3.2.1.1 Soil type and fertility

The soil is a moderately well drained, deep (> 1 m) 'Wakanui' silt loam (*Typic Eutrachept*, United States Department of Agriculture (USDA, 1985)), with a high water holding capacity of 150—200 mm/m depth. The site was previously under cropping (Table 3.1).

A soil test on the 3rd of August 2017, before ploughing showed moderate soil fertility to 0.15 m depth for all nutrients and to 1.20 m depth for mineral N (kg/ha) (Table 3.2). For mineral N, soil cores were taken at 0.15 m depth to 0.30 m and at 0.30 m depths thereafter to 1.20 m, to allow calculation of N at different layers. The amount of each soil nutrient was determined as 'MAF quick-test units' (Mountier et al. 1966) and converted into mg/kg dry soil (Table 3.2) using the following conversion factors: P×1.10; Ca, ×125; K, ×20.0; Mg, ×5.0; Na, ×5.0; S, × 1.0 (Chapman & Bannister 1994).

Table 3.1: Sowing and emergence dates, soil description and previous cropping history for the experimental site.

Parameter	Description
Sowing date	6 September 2017
Emergence (50%)	22 September 2017
Soil type	Wakanui silt loam
Soil Classification	Mottled Immature Pallic
Soil texture	Silty loam over loam
Drainage	Imperfectly drained
Mineral N to 1.2 m (kg/ha)	85
Previous cropping	
One year previous	Peas (*Pisum sativa* L.)
Two years previous	Barley (*Hordeum vulgare* L.)
Three years previous	Ryegrass (*Lolium perenne* L.)
Four years previous	Nui ryegrass (seed)

Basal fertilisers were applied at 300 kg/ha of 30% Potash superten (N:P:K:S:Ca; 0: 6.3:15:7.7:14) providing, 19.0 kg/ha P, 45.0 kg/ha K, 23.1 kg/ha S and 42.0 kg/ha Ca. Base fertiliser was applied on 23 August 2017, when the experimental site was marked out.

Table 3.2**:** Soil fertility test results (0—0.15 m) for the site for all nutrients[1], except mineral N (MN; 1.2 m depth). The optimum values are 'recommended' values for non-limited crop production (McLaren & Cameron 1996; Nicholls et al. 2012).

	pH	Olsen P	K	Ca	Mg	S	Na	Mineral N[2]
				mg/kg				kg/ha
Exp. site	5.7	21	120	1000	60	7	25	85
Optimum	5.8-6.2	20-25	120-200	1250-1500	40-50	10-15	50-75	150-200

[1]P=phosphorus, K=potassium, Ca=calcium, Mg=magnesium, S=sulphur and Na=sodium
[2]Measured to 1.2 m depth, all other minerals to 0.15 m depth.

3.2.2 Experimental design and treatments

The experiment was a randomised complete block design, replicated three times. The treatments consisted of the six wheat genotypes (Appendix 1.1) and two rates of N fertiliser supply (Section 3.2.2.2). The experiment was irrigated weekly, applying 25.0 mm of water each cycle.

3.2.2.1 Wheat genotype treatments

Six spring, modern wheat genotypes (Section 1.4; Appendix 1.1) were used in this study; five sourced from Plant & Food Research, Lincoln and one from PGG-Wrightson (Hay 2014; PGG-Wrightsons 2018). The genotypes were recommended by New Zealand wheat breeders to have a range of attributes that could enhance NUE (Section 1.4).

3.2.2.2 Nitrogen treatments

Two levels of N fertiliser supply (0 and 200 kg N/ha), were used with the optimum N fertiliser rate (200 kg/ha) estimated to be the amount necessary for non-limiting crop growth based on the 'Sirius' Wheat Calculator simulations (Armour et al. 2004). The choice of N treatments was to compare crop yield under limited and optimum N fertiliser supply and determine how these may affect the crop yield, nutrient (e.g. N) accumulation and partitioning. The N fertiliser was applied as urea (46% N) and was broadcast by a Solo Hand Spreader (SOLO Kleinmotoren GmbH. Stuttgart Straße 41. 71069 Sindelfingen, Germany). The N was applied as 100 kg N/ha at the start of tillering (GS21) and another 100 kg N/ha at the appearance of the second node (GS32), based on the Zadoks et al. (1974) growth scale (Section 3.3.1.2). A small (<10 mm) irrigation event followed the N fertiliser applications to dissolve the urea.

3.2.3 Cultural practices

3.2.3.1 Seedbed preparation and crop establishment

Land preparation commenced on 23 August 2017, and standard farm practices were followed to attain a suitable seedbed. The experiment was sown with a 9-row Øyjoord drill, with 0.15 m row spacing. There were 36 plots, which were 1.35 m wide and 12.0 m long.

A germination test before sowing showed ≥ 95% germination for all genotypes. This, together with the thousand grain weight (TGW, g) (Section 2.2), was used to determine seed rate per plot, to establish the standard 275 plants per m^2 (McCloy 1980; PGG-Wrightsons 2018). This figure is higher than the 150 plants per m^2 [sowing rate of 165 seeds per m^2] recommended for winter wheat (Stephen et al. 2005; Craigie et al. 2015), because spring sown wheat does not produce as many fertile tillers as winter wheat. The aim was to produce 600 ears per m^2 (Scott et al. 1977; Scott 1978; McCloy 1980; Hampton et al. 1981).

3.2.3.2 Herbicides, fungicides and pesticides

A prophylactic spray programme was applied to control pests, diseases and keep weeds infestation to minimum levels, and this was effective. Full details of the fungicides, herbicides and pesticides applied are shown in Table 3.3. All seeds were treated with Raxil®Ultra (fungicide) and Poncho® (insecticide) mix before sowing (Table 3.3). Poncho is an insecticide that provides a broad spectrum protection against attack by both above and below ground pests. Raxil®Ultra is a fungicide for the control of loose smut (*Ustilago tritici* (Pers.) Rostr.) and stinking smut (*Tilletia caries (*DC.) Tul.) of wheat. All other chemicals were applied as and when needed (Table 3.3).

Table 3.3: Agrichemicals applied during establishment and crop growth to different wheat genotypes grown at low and optimal nitrogen fertiliser supply at Lincoln, Canterbury, New Zealand in 2017–18 season.

Chemical group	Trade name	Active ingredient (a.i.)[1]	Rate of application (/ha)[2]	Date
Herbicide	Glean®	750 g/kg chlorsulfuron (WDG)	15 g	7 Sept. 2017
	Protugan®	500 g/L isoproturon (SC)	400 ml	7 Sept. 2017
	Quantum®	250 g/kg tribenuron methyl 250 g/kg thifensulfuron methyl	100 ml	7 Sept. 2017
	Hussar® OD	100 g/L iodosulfuron-methyl sodium	150 g	24 Oct. 2017
	Partner	280 g/L bromoxynil	1 l	24 Oct. 2017
Insecticides	Poncho®	600 g/litre clothianidin (SC)	0.6 ml/kg seed	6 Sept 2017
	Pirimor®	500 g/kg pirimicarb (WDG)	100 g	17 Oct. 2017
	Karate®	2.5g/L Lambda-cyhlothrin (EC)	40 ml	30 Oct. 2017
Fungicide	Raxil® Ultra	120 g/L tebuconazole (FSC)	0.21 ml/ kg seed	6 Sept. 2017
	Opus®	125g/L epoxiconazole	500 ml	30 Oct. 2017

[1]WDG= water disposable granule, SC=soluble concentrate, WS=water soluble, EC=emulsifiable concentrate, FSC=flowable suspension concentrate.
[2]Rates per ha, unless stated otherwise

3.3 Crop measurements

3.3.1 Crop establishment and canopy development

3.3.1.1 Seedling emergence

Crop establishment was monitored in a fixed 1.0 m length of two rows selected at random in each plot. Emerged seedlings were counted every 2 days until emergence was complete.

Emergence was defined as the time when at least 50% of the coleoptiles had emerged (Angus et al. 1980). The emergence date was determined retrogressively, when at least three of the six genotypes had reached 50% emergence. At least 50% of the seedlings had emerged two weeks after sowing, on 22 September 2017.

3.3.1.2 Crop development

Six contiguous plants were marked for non-destructive sampling from the third row of each plot. Using these six plants, the date at which a crop reached a given growth stage (GS) was assessed according to Zadoks et al. (1974) and Tottman et al. (1979). A GS was assigned when at least 50% of the main shoots, on at least three of the six contiguous plants were at that stage. Crop maturity was recorded when the entire green lamina area had senesced and <10% of the stem green area remained (Pask 2009). In the period leading up to anthesis, plot GS was assessed every 2-3 days to accurately determine the exact date of anthesis. As the crop matured, two sections of the plots [1.20 m long and 1.40 m wide] were covered with bird netting, 2.0 m above the ground from 12 November 2017 to harvest maturity, to prevent birds from eating the maturing grain to ensure accuracy of yield results.

3.3.2 Biomass accumulation and canopy development

Growth of plants may be expressed in a number of ways (Brown 1984), with the increase in height the most obvious manifestation. Increase in biomass is the most important aspect of crop growth and was the key measurement. All plant material harvested was stored in a cool room (at ~5°C) and then processed/ partitioned within 12 hours of harvests before drying to constant weight (Section 3.3.2.1). Details for the specific harvests are given in the following sections.

3.3.2.1 Seasonal biomass accumulation and partitioning

Dry matter accumulation was determined once before the first N fertiliser application at the start of tillering (GS21), on 23 October 2017 (31 days after emergence; DAE), and then at 10-12 day intervals after appearance of the flag leaf (GS39). The harvests prior to GS21 were from a 0.20 m^2 quadrat, and those between GS39 and GS55 (when 50% of inflorescent had emerged) were from a 0.30 m^2 quadrat. This was followed by 0.50 m^2 quadrat harvests from anthesis (GS65) to dough development (GS81). The final two harvests from GS85, from the

sections of the plots under netted cages (Section 3.3.1.2) were from a 1.0 m^2 quadrat. All harvests were done by hand. Subsamples of 250 g, 300 g and 500 g for fresh weight, for the harvests prior to GS65, between GS65 and GS85 (soft dough stage), and post GS85 [final 2 harvests], respectively, were taken from each plot and used to determine dry matter content (DM%) of the crop. For DM%, subsamples were dried in a forced air oven at 90°C, to a constant weight.

From GS39 onwards, 20 fertile main stem samples were selected at random from the harvest and partitioned into: green leaf lamina, green leaf sheath, true stem, ear (where applicable) and combined dead leaf lamina and/or sheath. The partitioned material was dried in a forced air oven at 60°C until constant weight for ~72 hours, and weighed. These samples were ground with a Cyclone Sample Mill (Udy Corporation, Fort Collins, Colorado, USA) to pass through a 1 mm screen and placed in plastic vial for storage. The ground samples were analysed for N yield (Section 3.5) and also mineral nutrient yield (Chapter 5).

Green leaf area was determined from the partitioned leaf lamina at each destructive harvest, using a Licor 3100 area meter (Licor Inc, NE, USA). This was used to calculate the GLAI (Section 2.2). Canopy senescence was calculated as the difference between the total number of leaves for each of the six marked plants and the number of green leaves still on the plant. Green leaves were counted twice weekly, throughout the growing season. The critical GLAI ($GLAI_{crit}$) was described as the GLAI values when crops intercept 90—95% of the incoming radiation (Brougham 1958). This range of GLAI indicates the point when canopies are close to total closure, which enables maximum growth rates due to near total light interception.

Values of GLAI expansion and senescence rates (LAER, LASR, respectively) in relation to thermal-time accumulation (Tt_{acc}, $m^2/m^2/°Cd$) (Section 3.6.1.1; Equation 3.2) were calculated from values between successive GLAI measurements. These measurements were carried out at key growth stages of tillering (GS21), stem development (GS31), flag leaf (GS39), anthesis (GS65), milk development (GS72) and soft dough growth stage (GS85). Negative values indicated senescence.

3.3.2.2 Harvest maturity stage

At harvest maturity, a 1.0 m^2 quadrat of above ground biomass (AGB) samples was cut and placed inverted into clean, dry paper sacks in the field to avoid grain loss during transport. Plants were harvested when all leaves were senesced, and when ≥ 90% of the stem was senesced. The low N treatments matured earlier, and were harvested 4 days (64°Cd) earlier than the 200 kg N/ha treatments. In the laboratory, the whole sample was air-dried to constant weight. Once dry, the sample was weighed and 20 undamaged stems were removed for further partitioning into leaf lamina, sheath, true stem, grain and non-grain (chaff) components. The rest of the sample was threshed in a Saatmeister mill (Kurt Pelz, Maschinenbau, Germany) to separate the grain from the chaff, after which the grain sample was further cleaned in the Rationel Kornservice A/S sample cleaner (Pfeuffer GMBH Kitzingen, Esbjerg, Denmark). The grain yield was adjusted to a standard 14% moisture content (86% DW; t/ha). The chaff DW was determined by subtracting the grain DW from ear DW. The total seed weight was recorded and then assessed for TGW (g) using a Numigral seed counter (Chopin Technologies, Paris, France). The percent moisture and hectolitre (hl; metric unit equal to 100 litres) test weight (kg/hl) of the grain (Appendix 1.1) were determined using a Dickey-john GAC500 XT moisture meter (Grainman corporation, FL, USA). The number of grains per ear and CHI were calculated from these data. Screenings (Sharma & Anderson. 2004) were separated out of a ∼250 g sub-sample of seed using a 2.0 mm screen.

3.4 Meteorological data and irrigation

Three neutron probe (NP; model 503DR Hydroprobe, Instro Tek Inc., Raleigh, NC, USA) access tubes [one in each rep, all located in 'Discovery' plots grown under 200 kg N/ha] and 12 time domain reflectometer rods (TDR; model CS650 Water Amount Reflectometers, Campbell Scientific Inc., Utah, USA) [all plots in Rep 1], were installed on 3 October 2017, to monitor soil moisture. These were used to follow soil moisture dynamics, in response to the overhead irrigation (25 mm per pass) applied once-weekly from the 4th of October 2017 to avoid crop water stress in the dry spring/summer period (Figure 3.1).

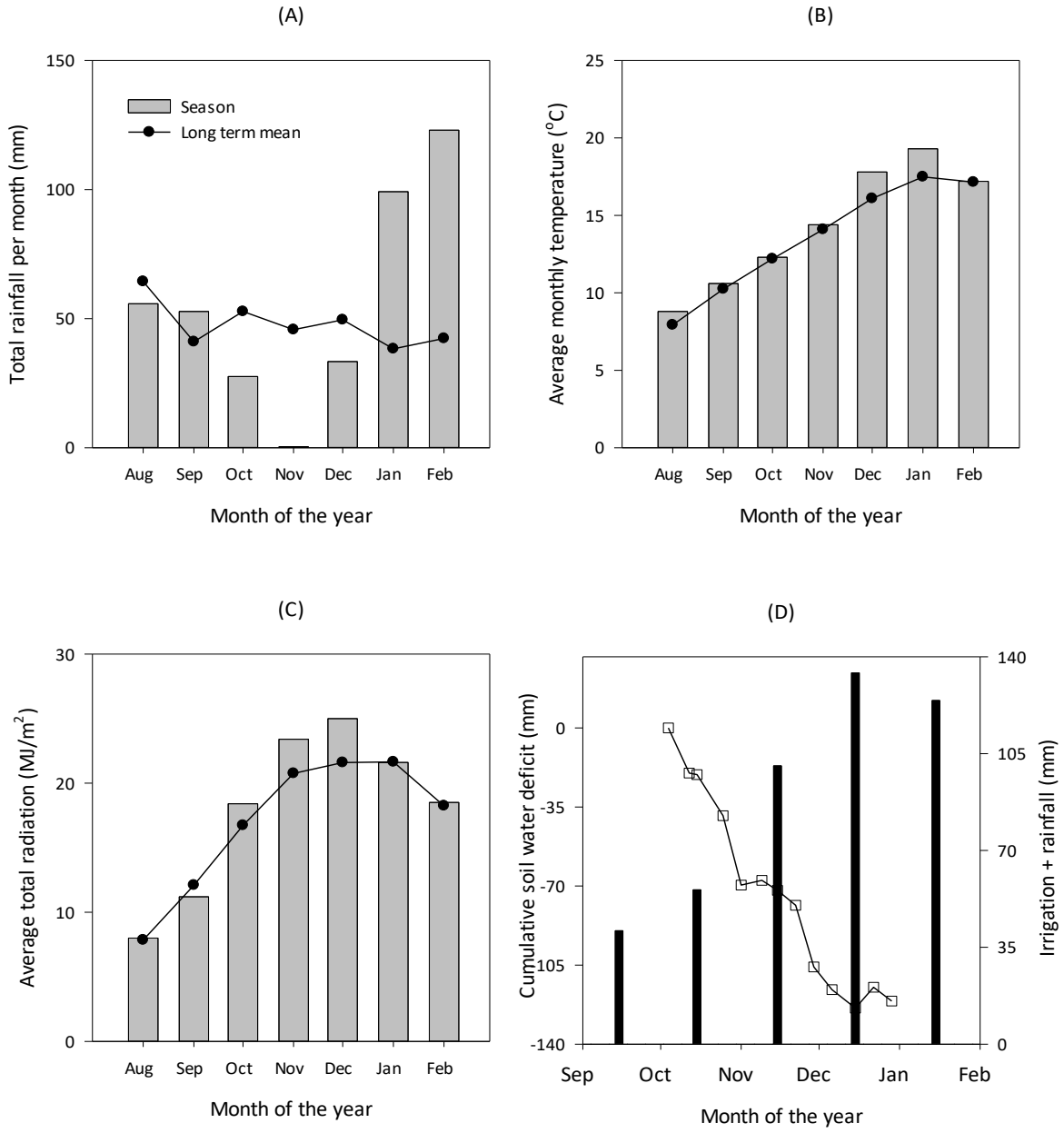

Figure 3.1: Monthly (A) rainfall (mm), (B) average temperature (°C) and (C) average total solar radiation (MJ/m^2) and (D) cumulative soil water deficit (mm, line) and total irrigation + rainfall (mm, bars) (Section 3.2.1) for the growing season (August 2017 to February 2018) and long-term mean (LTM) data at Lincoln, Canterbury, New Zealand. Long-term data are from 1970 to 2010 (NIWA 2019).

All weather variables were recorded throughout the crop-growing season. A rain gauge was installed on site to monitor both rainfall and irrigation over the season (Figure 3.1 D). Data from the NP and TDRs installed on site were used to calculate the accumulated moisture deficit (Figure 3.1.D) throughout the growing season. The seasonal rainfall was unevenly distributed, with November receiving less than 1% of the long-term mean (LTM), and January receiving 258% of the LTM (46% of the total seasonal rainfall), when the crops were

in the grain filling period. Therefore, all the irrigation was applied between sowing and the end of December 2017, the driest period.

The total rainfall for the experimental period (1 Sept 2017 – 31 Jan 2018) was 213 mm (Figure 3.1), with a Penman potential evaporation of 578 mm. The total deficit of 371 mm was partially alleviated with 250 mm of applied irrigation at 25 mm per week. The maximum soil moisture deficit of 121 mm in this experiment (Figure 3.1D) was less than the 262 mm critical deficit (deficit beyond which yield is reduced) reported for wheat in a nearby block (Jamieson et al. 1995). Furthermore, a water extraction depth of 1.30 m was reported previously (*Loc. cit*), thus it is reasonable to assume that the soil water deficit in this experiment did not affect wheat production.

3.5. Determination of nitrogen yield and partitioning

3.5.1 Nitrogen content

The N concentration (N%) for each of the ground samples (Section 3.3.2.1) was determined by automated dry combustion-gas chromatography with a Vario Max CN Macro Elemental Analyser (Elementar GmbH, Hanau, Germany). This method takes into account the total N contained in the plant including nitrates (Justes et al. 1994). Total N yield (kg/ha) was calculated as the product of DM yield (kg/ha) and the N% in the harvested crop (Muchow 1988; Subedi & Ma 2005).

3.5.2 Nitrogen fertiliser recovery

For the crop N uptake, there are two sources of N for the field grown wheat: mineralisation and applied N fertiliser (Pask 2009). The recovery of each of these sources was quantified by:

1. Soil N: the total soil mineral N is difficult to measure accurately over the period of crop growth. It was therefore assumed that the 0 kg N/ha treatment (N zero treatment) takes up all of the available soil N and has an AGN (Section 2.3.1) representative of the available soil mineral N at harvest (from both mineral N at sowing and mineralised N during the growing season).

2. Fertiliser N: the AFR (Equation 2.6) was calculated by the ‘difference’ or ‘N balance’ method (Foulkes et al. 1998).

3.5.3 Specific leaf nitrogen (SLN)

Common measures for expressing leaf N content are either area-based (g N/m^2) or mass-based (%) (Li et al. 2018). These represent the specific leaf N (SLN: the leaf N content per unit leaf area; g N/m^2) (Sinclair & Horie 1989) and leaf N concentration (N%), respectively. Leaf N% and SLN are interconverted via leaf mass per area (LMA, g/m^2) (Wright et al. 2004).

3.6 Statistical analyses

Biomass and N accumulation and their partitioning responses were analysed using a mixed model approach, fitted with a restricted maximum likelihood (REML) programme in Genstat 18^{th} edition. An estimate of the variation associated with treatment means was given by least significant difference (LSD) tests ($\alpha = 0.05$), with associated degrees of freedom (d.f.). Where values show $P = 0.1$, a trend is indicated in the text. Assumptions were checked via standard residual plots and logarithmic or square root transformations applied when needed. Fixed effects in the models were genotype, N fertiliser supply, date and all interactions. Random effects accounted for the position (block + column) within the field and a correlation structure was modelled for date to account for repeated measures. Where there was an interaction of treatments, the ratio between interaction and main effects F-statistic values (variance ratio, v.r.) was used to determine whether to concentrate on interaction term or main effects. Ratios greater than 10 meant the main effects were emphasised. Order was considered and largest *p-value* reported. Each variable was analysed separately. Unless otherwise stated, interactions are given in the text only when they are significantly different ($P \leq 0.05$).

In the current experiment, biomass and N partitioning into different organs, had similar patterns across the genotypes, irrespective of biomass yield and N fertiliser supply. Therefore, biomass and N partitioning of the highest and lowest yielding genotypes have been used to show the relationships in this chapter. The results for the four other genotypes are in Appendix 3.1 & 3.2. Where there was an interaction, all genotypes and N fertiliser supply treatments are described, and where only the genotype was significant, then the highest and lowest yielding genotypes are described in the text. A functional growth analysis using a Maximum Likelihood Programme (MLP) from Rothamsted Experimental Station, UK

(Ross et al. 1987) was used. Generalised logistic curves were used to describe biomass accumulation of the crops as shown in Equation 3.1 (Gallagher & Biscoe 1978b):

$$Y = C/ (1.0 + T \exp (-b(x-m)))^{1/T} \quad \text{Equation 3.1}$$

Where Y = the yield

C is the final (maximum) above ground dry matter

and *T, b* and *m* are constants.

3.6.1 Calculations

3. 6.1.1 Crop development rates

To evaluate crop development rates, thermal time accumulation (Tt_{acc}, °Cd) was calculated from 50% emergence as Equation 3.2 (Morrison et al. 1989; Ritchie et al. 1998):

$$Tt_{acc} = \sum_i^n [(T_a - T_b)] \quad \text{Equation 3.2}$$

Where T_a is average daily temperature, and T_b is the base temperature (0°C) (Lafond & Baker 1986; Jamieson et al. 1998). The Tt_{acc} values were calculated daily from the beginning (*i*) to the end (*n*) of a given phenological stage, with a minimum value of zero (Morrison et al. 1989; Hodges 1991).

An empirical two-stage piecewise function was fitted to analyse the emergence response to T_t (Section 3.7.1), to describe the linear relationship between the start of emergence to 50% emergence; and then the curvilinear relationship thereafter to full emergence.

3.6.1.2 Nitrogen recovery and efficiencies

The NHI (Equation 2.2) (Foulkes et al. 2009b), AFR (Equation 2.6) (Foulkes et al. 1998), NUE and its components (Equations 2.3-2.5) (Moll et al. 1982; Ciampitti et al. 2013) are fully described in Sections 2.3.1 and 2.3.3.

3.6.1.3 Post-anthesis N remobilisation

Nitrogen remobilisation (NR; kg/ha) is the amount of N in the crop or crop components at anthesis which is not recovered in the crop non-grain components (straw + chaff) at harvest (Cox et al. 1986), and is calculated by the 'apparent remobilisation' method:

NR = N yield at anthesis – N yield in straw + chaff at harvest maturity Equation 3.3

3.6.1.4 Post-anthesis N uptake

Post-anthesis N uptake (PANU; kg/ha) is the amount of N in the crop at harvest which was not present in the crop at anthesis and is assumed to be the result of continued soil N uptake post anthesis (Cox et al. 1986):

PANU = AGN at harvest – AGN at anthesis Equation 3.4

Note: Estimation of both NR and PANU using the 'apparent remobilisation' method is subject to large experimental errors due to combining of data from two different sampling dates (Kichey et al. 2007). The determination does not quantify any loss of N through volatilisation or mobilisation into the roots

Partial regression analysis was performed for PANU as a dependent variable and N yield at anthesis and grain N (N_g) yield as independent variables (Dhugga & Waines 1989).

3.6.1.5 Post-anthesis N remobilisation efficiency

Nitrogen remobilisation efficiency (NRE, %) is the fraction of N in the crop or crop organs at anthesis which is not recovered in the crop non-grain component at harvest (Cox et al. 1986) as a percentage:

NRE = (NR / N yield at anthesis)*100 Equation 3.5

3.7 Results

3.7.1 Plant establishment

Seedling counts were related to Tt_{acc} based on T_b of 0 (Section 3.6.1.1). Seedling emergence reached 50% (Section 3.3.1.1) after Tt_{acc} of 168°Cd (Figure 3.2) at an average of 129±14.0 seedlings/m^2.

Time to 50% emergence differed (P = 0.03) among the genotypes, with three ('Discovery', PFR-2021 and 'Reliance') of the six genotypes (50%; Section 3.3.1.1) emerging earlier (P < 0.05) (168°Cd) than the others, which averaged 179°Cd (Figure 3.2). All genotypes attained a similar initial population of 254±16.0 seedlings/m^2 (Section 3.2.3.1) at 200°Cd, and therefore no effect of initial population on results is reported in this thesis.

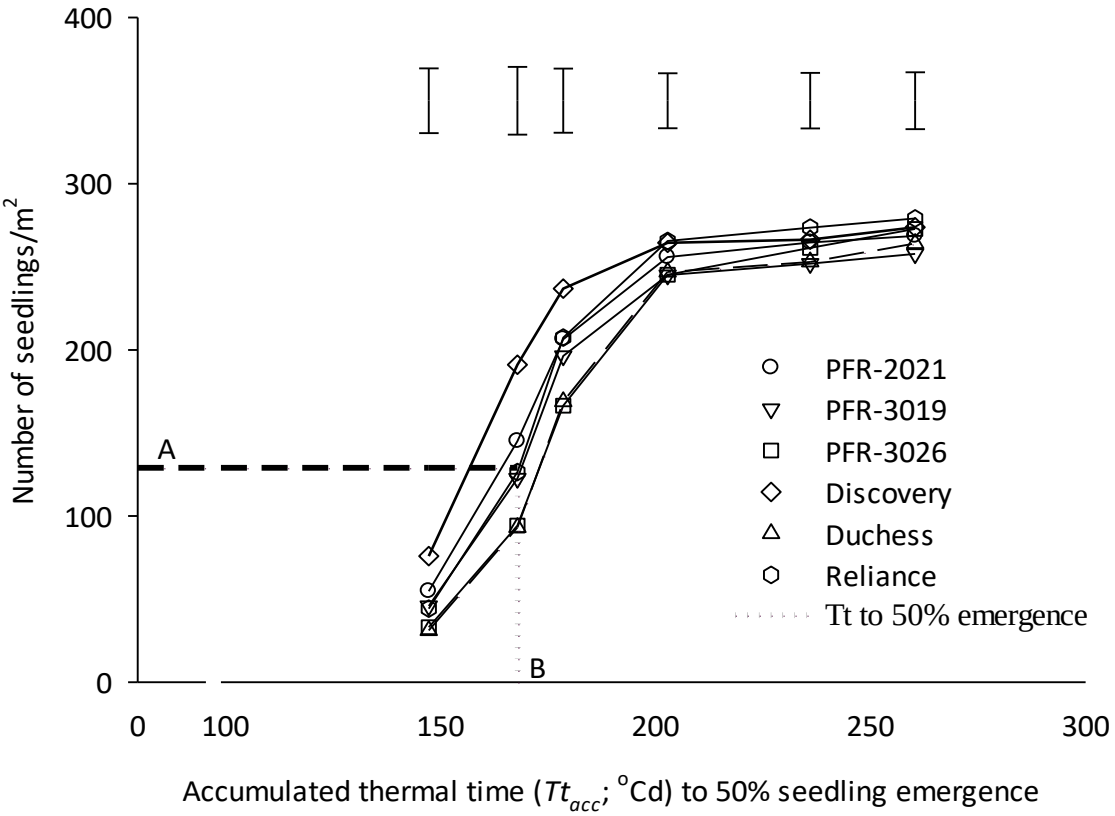

Figure 3.2: The number of seedlings emerging over accumulated thermal time (Tt_{acc}; °Cd) for different spring wheat genotypes sown on 6 September 2017 at Lincoln, Canterbury in New Zealand in 2017-18 growing season. Vertical bars are least significant difference ($LSD_{5\%}$), and dotted lines represent the number of seedlings (long dash) and the accumulated T_t at 50% emergence (dotted lines), respectively.

3.7.2 Crop harvest index (CHI)

The CHI was unaffected (P = 0.25) by N fertiliser supply, at an average of 0.55±0.01 (Table 3.4; Figure 3.3 A). However, CHI differed (P = 0.01) among genotypes and PFR-2021, PFR-3026 and 'Reliance' had higher CHI, at an average of 0.57±0.02 compared with 0.53 for the other three genotypes.

Table 3.4: Crop harvest index for the six wheat genotypes grown with 0 or 200 kg N/ha at Lincoln, New Zealand, during 2017-18 season

	N treatment[1]		
Genotype	0 kg N/ha	200 kg N/ha	Mean[2]
PFR-2021	0.58_{a}	0.58_{a}	0.58_{a}
PFR-3019	0.54_{b}	0.54_{b}	0.54_{bc}
PFR-3026	0.58_{a}	$0.55a_{b}$	0.57_{ab}
'Discovery'	0.53_{bc}	0.54_{b}	0.54_{bc}
'Duchess'	0.50_{c}	0.55_{ab}	0.52_{c}
'Reliance'	0.56_{ab}	0.59_{a}	0.57_{ab}
Mean	0.55	0.56	0.55
Significance: P value ($LSD_{5\%}$)			
N fertiliser supply	0.25 (0.02)		
Genotype (G)	0.02 (0.03)		
N*G	0.34 (0.05)		

[1]Soil N to 1.2 m depth was 85 kg/ha

[2]Means with letter subscripts in common, within a column are not different at α = 0.05

3.7.2.1 Above ground biomass (AGB) and grain yield

Total AGB yield increased (P < 0.001) with increasing N fertiliser supply from an average of 12.0±0.40 t/ha for the 0 kg N/ha treatment to 15.8 t/ha when 200 kg N/ha was applied. Total AGB yield showed a trend (P = 0.09) to differ among genotypes (Table 3.5). Mean AGB yield for 'Discovery' (15.0±0.65 t/ha) was higher than the 12.9 t/ha for 'Reliance'. There were no difference among the other genotypes with either 'Discovery' or 'Reliance'.

For grain yield, there was an interaction (P = 0.05) between N fertiliser supply and genotype. This was caused by PFR-2021 which showed both the highest grain yield at 200 kg N/ha of 9.40±0.30 t/ha and the lowest yield at 0 kg N/ha of 5.98 t/ha (Table 3.5). At 200 kg N/ha, grain yield was higher (P<0.001) for PFR-2021 and 'Discovery', at an average 9.44±0.30 t/ha, intermediate for PFR-3026 at 8.66 t/ha compared with the 8.34±0.30 t/ha for the other genotypes. At 0 kg N/ha, grain yield was higher (P < 0.001) for PFR-3026 at 7.35 t/ha and intermediate for 'Discovery' (6.72 t/ha), compared with 6.26±0.30 t/ha for the other genotypes.

Table 3.5: Mean[1] total above ground biomass (AGB; t/ha) and grain yield (t/ha) at harvest maturity for six wheat genotypes grown with 0 or 200 kg N/ha at Lincoln, Canterbury, New Zealand, during 2017-2018 season.

Genotype	Yield (t/ha)					
	Total AGB			Grain		
N fertiliser (kg N/ha)	0	200	Mean	0	200	Mean
PFR-2021	10.9_{b}	16.3_{a}	13.5_{ab}	5.98_{b}	9.40_{a}	7.81_{ab}
PFR-3019	12.1_{a}	15.6_{ab}	13.8_{ab}	6.45_{ab}	8.46_{b}	7.51_{ab}
PFR-3026	12.3_{a}	15.7_{ab}	14.0_{ab}	7.35_{a}	8.66_{ab}	7.89_{a}
'Discovery'	12.4_{a}	17.6_{a}	15.0_{a}	6.72_{ab}	9.47_{a}	8.03_{a}
'Duchess'	12.8_{a}	14.9_{b}	13.9_{ab}	6.42_{ab}	8.11_{b}	7.25_{b}
'Reliance'	11.3_{b}	14.5_{b}	12.9_{b}	6.19_{ab}	8.46_{b}	7.46_{ab}
Mean	12.0_{b}	15.8_{a}	13.9	6.52_{b}	8.77_{a}	7.64
Significance: P value ($LSD_{5\%}$)						
N fertiliser supply	< 0.001 (0.78)			< 0.001 (0.35)		
Genotype (G)	0.09 (1.34)			0.05 (0.61)		
N*G	0.13 (1.90)			0.05 (0.86)		

[1]Means with letter subscripts in common, within a column are not different at α = 0.05

Grain yield was moderately related to leaf lamina biomass (R^2 = 0.62) and number of grains/m^2 (R^2 = 0.67) (Figure 3.3). There was no relationship (R^2 = 0.001) between grain yield and TGW. However, when the relationships were separated on N fertiliser supply, there was a moderate relationship (R^2 = 0.67) for the 200 kg N/ha crops and none (R^2 = 0.001) for the 0 kg N/ha crops.

3.7.2.2 Screenings

There was an interaction (P = 0.01) between N fertiliser supply and genotype (Table 3.6) for screenings, as they increased (P < 0.001) with increasing N fertiliser supply for all genotypes except for PFR-2021 and 'Discovery'. Screenings differed (P < 0.001) among the genotypes, being highest for 'Duchess' at 4.28% for the 0 kg N/ha and 5.39% for the 200 kg N/ha treatments, compared with the other genotypes. 'Discovery' and PFR-2021 had the lowest screenings at both N fertiliser supply rates.

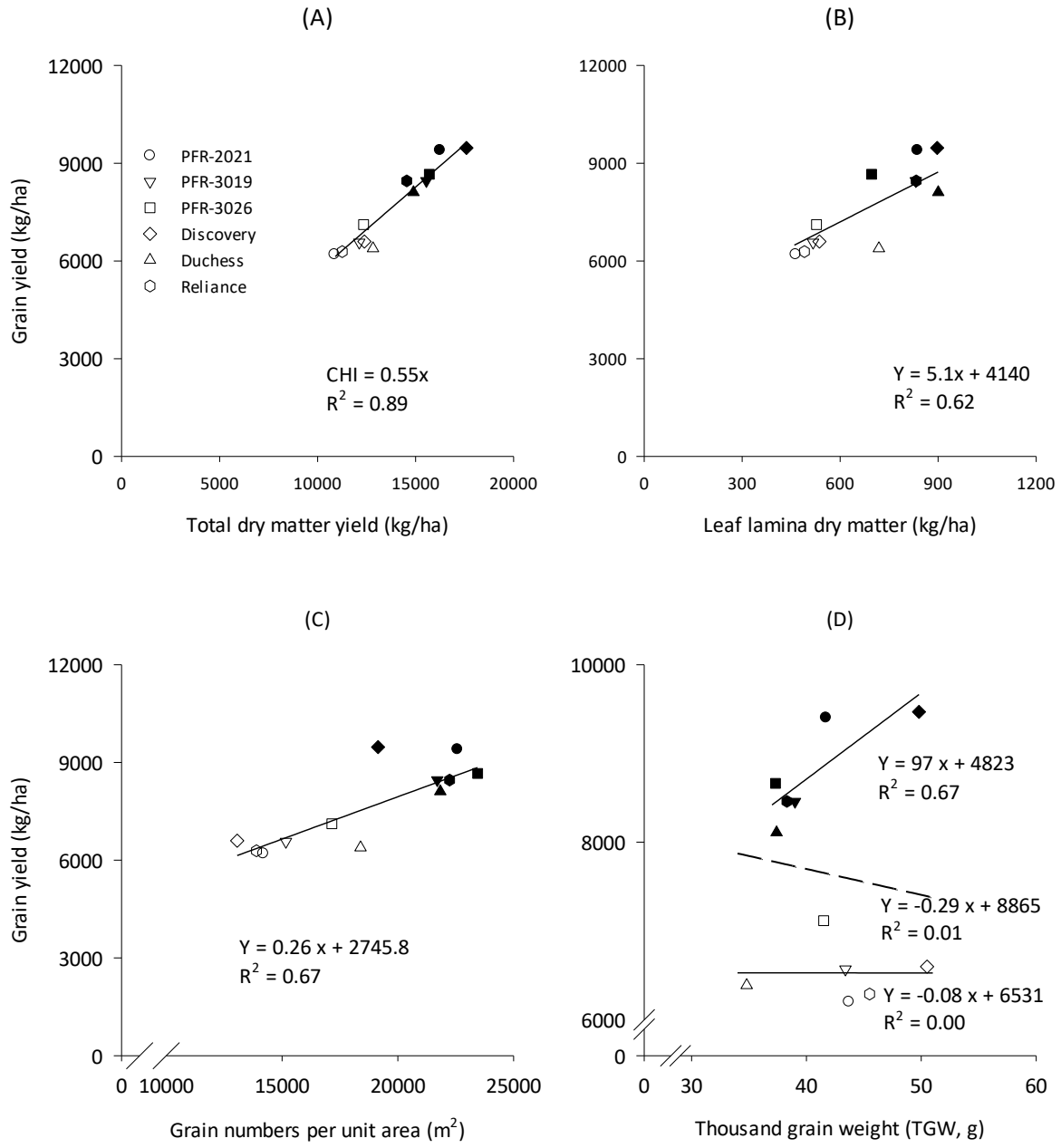

Figure 3.3: Relationship between grain yield (kg/ha) and: (A) total dry matter yield [kg/ha] (B) leaf lamina dry matter yield [kg/ha], (C) number of grains per ground area (m^2) and (D) thousand grain weight (TGW, g) [the dotted line represent the combined data]. Open symbols are the 0 kg N/ha and closed symbols are the 200 kg N/ha treatments. Respective genotypes are given in Figure 3.3 A.

The low screening (Table 3.6) for the moderate to high TGW genotypes [PFR-2021, 'Reliance', 'Discovery'] (Figure 3.4) resulted in a negative relationship (Y = -0.25x + 12.8; R^2 = 0.60) between screening and TGW.

3.7.2.3 Plant height

Plant height was unaffected (P = 0.17) by N fertiliser supply (Table 3.6) at an average of 69.0±0.54 cm. However, plant height differed (P < 0.001) among the genotypes, being taller for the moderate (PFR-3026) and high ('Discovery', PFR-2021) yielding genotypes at an average of 70.4±0.94 cm, compared with the 67.5 cm for the low yielding genotypes.

Table 3.6: Mean[1] plant height (cm), chaff biomass (t/ha) and screenings (%) for the six wheat genotypes grown with 0 or 200 kg N/ha at Lincoln, New Zealand, during 2017-18 season

Genotype	Other yield parameters					
	Plant height		Chaff (t/ha)		Screenings (%)	
N fertiliser (kg N/ha)	0	200	0	200	0	200
PFR-2021	70.7_{a}	70.7_{a}	1.51_{a}	2.12_{a}	1.03_{b}	1.37_{cd}
PFR-3019	67.7_{b}	66.0_{b}	1.85_{a}	2.32_{a}	1.24_{b}	3.09_{b}
PFR-3026	70.0_{a}	69.7_{a}	1.63_{a}	2.28_{a}	1.58_{b}	2.25_{bc}
'Discovery'	71.0_{a}	70.3_{a}	1.53_{a}	1.84_{a}	1.10_{b}	0.73_{d}
'Duchess'	66.7_{b}	67.0_{b}	1.90_{a}	2.03_{a}	4.28_{a}	5.39_{a}
'Reliance'	70.0_{a}	67.7_{b}	1.60_{a}	1.68_{b}	1.22_{b}	4.37_{ab}
Mean	69.3_{a}	68.6_{a}	1.67_{b}	2.04_{a}	1.74_{b}	2.87_{a}
Significance: P value ($LSD_{5\%}$)						
N fertiliser supply	0.17 (1.13)		0.001 (0.21)		< 0.001 (0.55)	
Genotype (G)	< 0.001 (1.95)		0.14 (0.37)		< 0.001 (0.95)	
N*G	0.70 (2.76)		0.48 (0.52)		0.014 (1.34)	

[1]Means with letter subscripts in common, within a column are not different at α = 0.05

3.7.3 Yield components

3.7.3.1 Thousand grain weight (TGW, g)

There was an interaction (P = 0.04) between the N fertiliser supply and genotype for the TGW (Figure 3.4). This was because TGW was higher (P = 0.003) for the 0 kg N/ha compared with the 200 kg N/ha treatments, for PFR-3019, PFR-2021 and 'Reliance', and unaffected by N fertiliser supply for the other genotypes.

The TGW differed (P < 0.001) among the genotypes. 'Discovery' had the highest TGW for both N fertiliser rates at a mean of 50.0±1.40 g (Figure 4). 'Duchess' had the lowest TGW (35.0 g) for the 0 kg N/ha crops. The other four genotypes were intermediate at 43.5±1.40 g. When 200 kg N/ha was applied PFR-2021 was intermediate (41.7 g), compared with the 38.0±1.40 g for the other three genotypes.

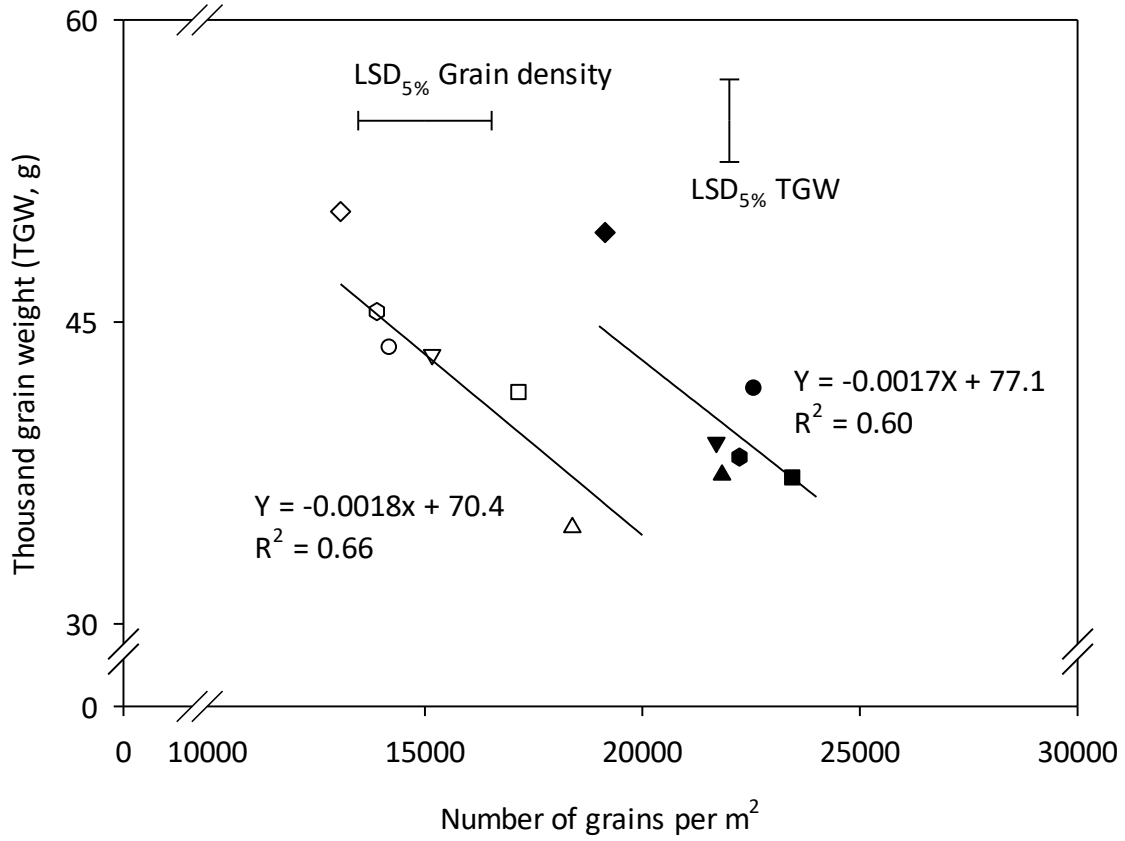

Figure 3.4: Relationship between thousand grain weight (TGW; g) and the number of grain per unit area for six wheat genotypes (● PFR-2021, ▼ PFR-3019, ■ PFR-3026, ◆ 'Discovery', ▲'Duchess' and ⬣ 'Reliance') grown with 0 kg N/ha (open symbols) or 200 kg N/ha (closed symbols) at Lincoln, Canterbury in 2017-18 season. Horizontal and vertical bars are the least significant differences ($LSD_{5\%}$).

3.7.3.2 Number of grains per unit area (density)

The number of grains/m^2 (grain density) differed (P = 0.012) among the genotypes (Figure 3.4). 'Discovery' had the lowest grain density at both N fertiliser rates: 13,000 grains/m^2 for the 0 kg N/ha crops and 19,200 grains/m^2 when 200 kg N/ha was applied. At 0 kg N/ha, PFR-3026 and 'Duchess' had the highest grain density, at 17,770±740 grains/m^2 (Figure 3.4). When 200 kg N/ha was applied, there were no differences among the other five genotypes at 22,360±740 grains/m^2.

Grain density increased (P < 0.001) with increasing N fertiliser supply from a mean of 15,300±430 grains/m^2 for the 0 kg N/ha crops to 21,800 grains/m^2 when 200 kg N/ha was

applied. This, together with the decrease in TGW with increasing N fertiliser supply, gave a negative but moderate ($R^2 \geq 0.60$) relationship between these two yield components (Figure 3.4).

3.7.4 Canopy development

3.7.4.1 Green leaf area

The GLAI (Section 2.2) was slow during crop establishment and was ~0.50 m^2/m^2 at the start of tillering (385°Cd) (Figure 3.5). The increase in GLAI was most rapid between 385 and 794°Cd for the 0 kg N/ha crops, but up to 960°Cd when 200 kg N/ha was applied (Figure 3.5). The GLAI was stable between 960 and 1,330°Cd and within the $GLAI_{crit}$ (canopy closure; Section 3.3.2.1) for the 200 kg N/ha crops, while also stable for the 0 kg N/ha crops but below $GLAI_{crit}$. Thereafter, GLAI decreased rapidly to about 0.07 m^2/m^2 for the 0 kg N/ha crops and 0.60 m^2/m^2 for the 200 kg N/ha crops at 1,730°Cd (GS86), when measurements ceased.

At all times, the average GLAI was higher for the optimum N fertiliser supply crops (Figure 3.5 A), even during the period when all crops were decreasing after peak GLAI. This was attributed to their faster LAER (Section 3.3.2.1; Table 3.7) compared with the low N crops. The GLAI also differed among the genotypes between stem elongation (GS31; 530°Cd) (Table 3.7) and hard dough development stage (GS87; 1,510°Cd for the 0 kg N/ha or 1,730°Cd for the 200 kg N/ha) or physiological maturity (Tottman et al. 1979)), the period leading to peak GLAI for both N fertiliser supply rates, and covering the duration above $GLAI_{crit}$ for the optimum N fertiliser crops. The GLAIs for 'Reliance' and PFR-3019 were consistently lower than the other genotypes for both N fertiliser supply rates during this period, while 'Discovery' and PFR-2021 had higher GLAI at most measurement times for both N fertiliser rates, and PFR-3026 was intermediate. The maximum GLAI was 2.65 m^2/m^2 for 'Duchess' for the unfertilised crops and 3.73 m^2/m^2 ('Discovery') when optimum N fertiliser was applied, at 960°Cd.

Peak GLAI (Figure 3.5) was within the $GLAI_{crit}$ value of 3.0 – 3.70 m^2/m^2 for all fertilised crops, while none of the genotypes attained the $GLAI_{crit}$ for the non-fertilised crops. The

duration above the $GLAI_{crit}$, at optimum N fertiliser supply was longer (~650°Cd) for 'Discovery' and PFR-2021 compared with ~350°Cd for the other genotypes.

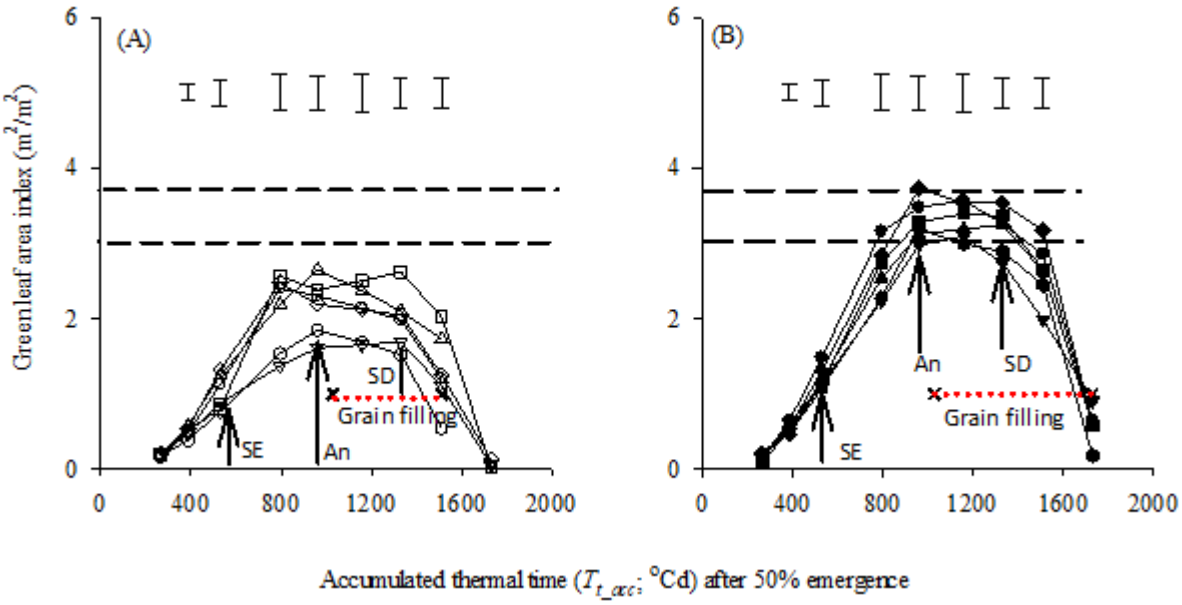

Figure 3.5: Green leaf area index (GLAI) against accumulated thermal time (Tt_{acc}; °Cd) after 50% emergence for six wheat genotypes (○ PFR-2021, ▽ PFR-3019, □ PFR-3026, ◇ 'Discovery', △ 'Duchess' and ⬡ 'Reliance') grown with: (A) 0 kg N/ha and (B) 200 kg N/ha at Lincoln, Canterbury in 2017-18 season. Horizontal long dash lines represents the critical GLAI ('canopy closure') of 3.0–3.70 m^2/m^2 (Hipps et al. 1983), dotted red lines represent the grain-filling period and the vertical bars are the least significant differences ($LSD_{5\%}$). Different growth stages: stem elongation (SE), anthesis (An) and soft dough (SD) (Table 3.7).

3.7.4.2. Leaf area expansion rate (LAER) and senescence rate (LASR)

The LAER differed ($P \leq 0.05$) among the genotypes at all growth stages up to flag leaf (Table 3.7). During the pre-tillering stage, 'Discovery' and 'Duchess' expanded ~35% faster (0.0017±0.00011 $m^2/m^2/°Cd$) than 'Reliance' and PFR-3026 (0.0011±0.00011), while the other genotypes were intermediate at 0.0015±0.00011. At stem elongation, 'Discovery' and PFR-2021 had faster LAER at 0.0045±0.0005 $m^2/m^2/°Cd$ compared with 0.0031±0.0005 $m^2/m^2/°Cd$ for the other genotypes. At flag leaf, the LAER for 'Discovery', PFR-2021 and 'Duchess' was faster at 0.0080±0.00041 $m^2/m^2/°Cd$, compared with 0.0057±0.00041 $m^2/m^2/°Cd$ for the other genotypes.

Table 3.7: The rate of green leaf area expansion (LAER) between successive GLAI measurements at key growth stages for six wheat genotypes grown with and without nitrogen (N) fertiliser (kg/ha) at Lincoln, New Zealand, in the 2017–18 season, in relation to thermal time (m^2/m^2/°Cd). Negative values represent leaf area senescence rate (LASR).

Treatments		LAER between successive measurements[1, 2]					
N fertiliser	Genotype	0–1	1–2	2–3	3–4	4–5	5–6
0 kg/ha	PFR-2021	0.0013_b	0.0041_a	0.0073_a	-0.0003_a	-0.0010_a	-0.0030_a
	PFR-3019	0.0013_b	0.0019_c	0.0037_c	0.0007_a	-0.0004_a	-0.0021_a
	PFR-3026	0.0011_{ac}	0.0026_{bc}	0.0046_{bc}	-0.0004_a	-0.0008_a	-0.0011_a
	'Discovery'	0.0019_a	0.0037_{ab}	0.0064_a	-0.0008_a	-0.0006_a	-0.0028_a
	'Duchess'	0.0020_a	0.0030_b	0.0060_{ab}	0.0013_a	-0.0014_a	-0.0030_a
	'Reliance'	0.0010_c	0.0025_{bc}	0.0048_{bc}	0.0009_a	-0.0008_a	-0.0025_a
	Mean	*0.0014_a*	*0.0030_b*	*0.0055_b*	*0.0002_b*	*-0.0007_a*	*-0.0024_b*
200 kg/ha	PFR-2021	0.0017_a	0.0054_a	0.0101_a	0.0009_a	-0.0005_a	-0.0038_a
	PFR-3019	0.0015_{ab}	0.0035_b	0.0066_{ab}	0.0004_a	0.0009_a	-0.0026_a
	PFR-3026	0.0013_{bc}	0.0033_b	0.0062_c	0.0005_a	0.0007_a	-0.0022_a
	'Discovery'	0.0014_{bc}	0.0049_a	0.0099_a	0.0005_a	-0.0008_a	-0.0041_a
	'Duchess'	0.0013_{bc}	0.0043_{ab}	0.0085_b	0.0007_a	0.0007_a	-0.0030_a
	'Reliance'	0.0012_c	0.0039_{ab}	0.0081_{ab}	0.0006_a	-0.0011_a	-0.0033_a
	Mean	*0.0014_a*	*0.0042_a*	*0.0082_a*	*0.0006_a*	*0.00001_a*	*-0.0032_a*
Significance[2]:							
N fertiliser supply (N)		ns	***	***	*	ns	*
Genotype (G)		*	**	***	ns	ns	ns
N*G		ns	ns	Ns	ns	ns	ns

[1]Means with letter subscripts in common, within a column are not different at $\alpha = 0.05$

[1] 0, emergence; 1, tillering; 2, stem elongation; 3, flag leaf; 4, anthesis; 5, milk development and 6, soft dough

[2] * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and ns = non-significant

During vegetative growth stages, LAER increased ($P < 0.001$) with increasing N fertiliser supply at all stages except the tillering stage (Table 3.7). For example, at stem elongation (542°Cd), LAER increased from 0.003±0.00027 m^2/m^2/°Cd for the 0 kg N/ha crops to 0.0042 m^2/m^2/°Cd when 200 kg N/ha was applied. Similarly, at flag leaf (714°Cd), LAER increased from 0.0055±0.00041 for the 0 kg N/ha crops to 0.0082 m^2/m^2/°Cd when 200 kg N/ha was applied. The LASR represented by the period after anthesis (3–4; Table 3.7), commenced earlier for the low N crops, but was faster for the optimum N crops at the final measurement. The LASR did not differ ($P \geq 0.28$) among the genotypes.

3.7.5 Biomass accumulation and partitioning

The pattern of biomass partitioning among the genotypes was not different (P = 0.25), irrespective of biomass yield or N fertiliser supply (Figure 3.6). Therefore the highest and lowest yielding genotypes ('Discovery' and 'Reliance', respectively) have been used to show the main effects (Figure 3.6), while the results for the other genotypes are shown in Appendix 3.1 & 3.2 (Section 3.6).

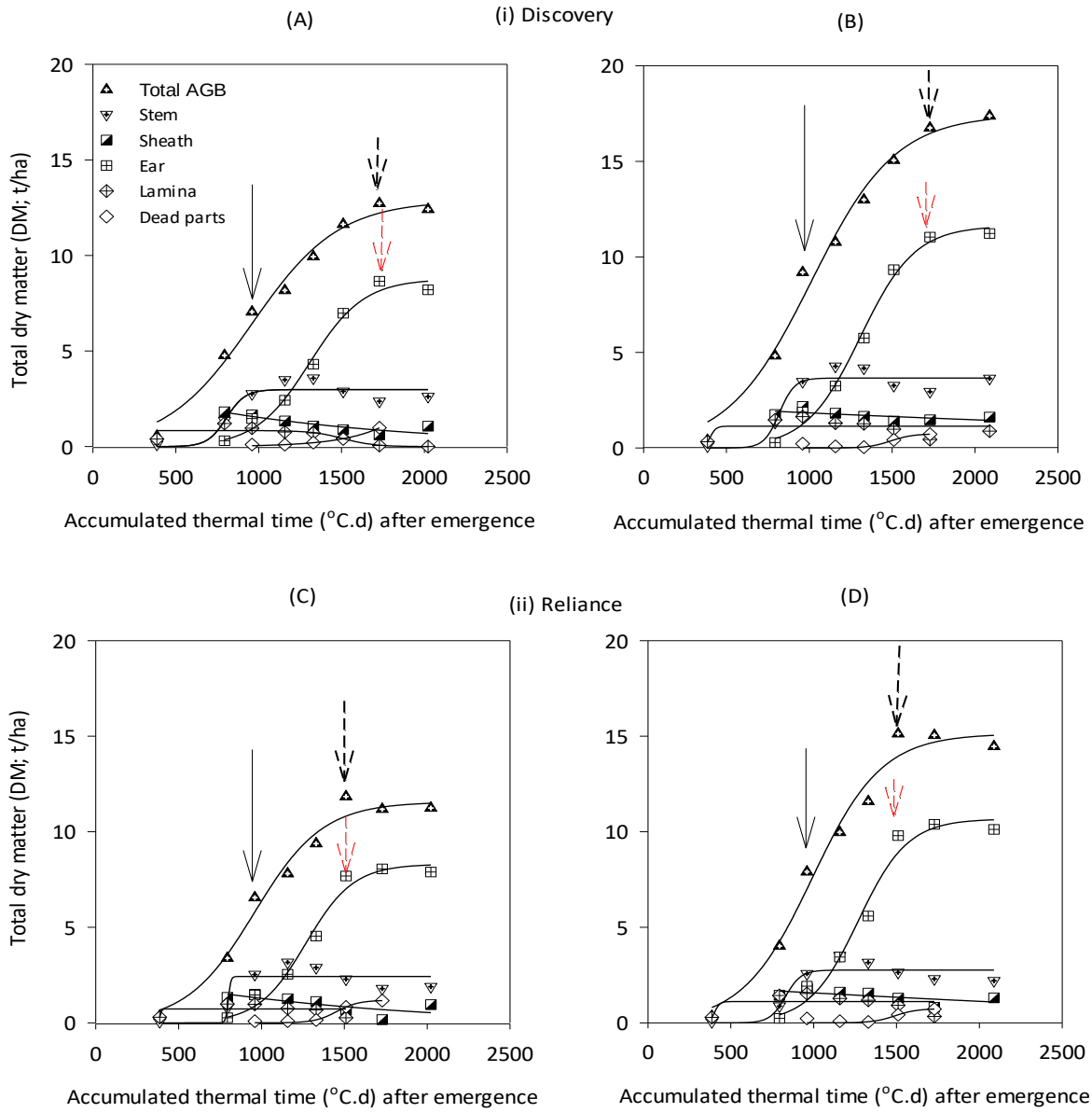

Figure 3.6: Relationship between mean accumulated dry matter (t/ha) for the different plant organs (see Key in Figure 3.6 A) and accumulated thermal time ($Tt_{acc,}$ °Cd) for (i) 'Discovery' and (ii) 'Reliance', grown with 0 kg N/ha (A & C) or 200 kg N/ha (B & D), at Lincoln, Canterbury, New Zealand. The solid and dotted arrows show anthesis and the physiological maturity (GS86), respectively [black, total AGB and red, ear biomass]. Relationships for the other four genotypes are shown in Appendices 3.1.

Time to anthesis was unaffected by N fertiliser supply or genotype, and averaged 960°Cd after 50% emergence (Figure 3.6, Appendices 3.1 and 3.2). Accumulated biomass at anthesis averaged 8.20±0.31 t/ha for 'Discovery' and 7.24±0.31 t/ha for 'Reliance' (Figure 3.6). This increased to 15.0±0.65 for 'Discovery' and 12.9 t/ha for 'Reliance' at harvest maturity (Table 3.5).

Time to physiological maturity (GS87), differed ($P < 0.05$) among the genotypes (Figure 3.6, Appendices 3.1 and 3.2), but was unaffected by N fertiliser supply. At both N fertiliser levels, three genotypes ['Duchess', PFR-3019, and 'Reliance'] reached physiological maturity at ~1,510°Cd, compared with ~1,730°Cd for the other genotype ('Discovery', PFR-2021 and PFR-3026). Delayed physiological maturity, meant ~220°Cd longer grain filling period (red dotted lines; Figure 3.5; 3.6, Appendices 3.1) for these genotypes, resulting in higher grain yields (Table 3.5). Harvest maturity was 64°Cd earlier ($P < 0.05$) for the 0 kg N/ha treatments, at 2,022°Cd after 50% emergence compared with the N fertilised crops (Figure 3.6).

Growing season biomass yield differed ($P < 0.001$) with N fertiliser supply at all sampling dates (Figure 3.6, Appendices 3.1 and 3.2). The low N crops accumulated biomass at 10.3±0.4 kg DM/ha/°Cd compared with 12.3 kg DM/ha/°Cd for the 200 kg N/ha crops. There were strong linear relationships ($R^2 \geq 0.96$) between dry matter accumulation and Tt_{acc} during the linear phase of growth [from 790°Cd to 1,510°Cd (0 kg N/ha) and 1,730°Cd (200 kg N/ha)] (Figure 3.6; Appendix 3.1 and 3.2). However, the slopes for the different genotypes did not differ ($P = 0.89$), with an average of 11.3 kg DM/ha/°Cd (Appendix 3.2). This meant the biomass differences among the genotypes were due to the differences in GLAI and grain filling period (Figure 3.5).

The AGB at anthesis was on average 55±5.0% of the total biomass at harvest maturity (Figure 3.6), did not differ ($P = 0.54$) among genotypes, but decreased ($P < 0.001$) from 59±2.0% for the low N crops to 51.0% at optimum N fertiliser supply.

3.7.5.1 Grain biomass

Ears started developing after about 750°Cd (Figure 3.6); when the crop had accumulated 4.86±0.28 t/ha for both N fertiliser rates for 'Discovery', and 3.40 t/ha for the low N and

4.10 t/ha for the optimum N fertiliser supply for 'Reliance'. The relationship between the ear biomass accumulation (t/ha) (Figure 3.6) and Tt_{acc} (°Cd) had an initial lag phase, before it increased rapidly over the linear phase.

The changes in the ear biomass yield (Figure 3.6) were mostly a result of increased grain dry matter (Figure 3.7). Therefore only grain dry matter is considered here (Table 3.8). Grain yield was closely related (R^2= 0.96) to the ear biomass yield and accounted for 82.0% of the ear biomass yield (Figure 3.7), and 55.0% of the total AGB (Figure 3.3 A).

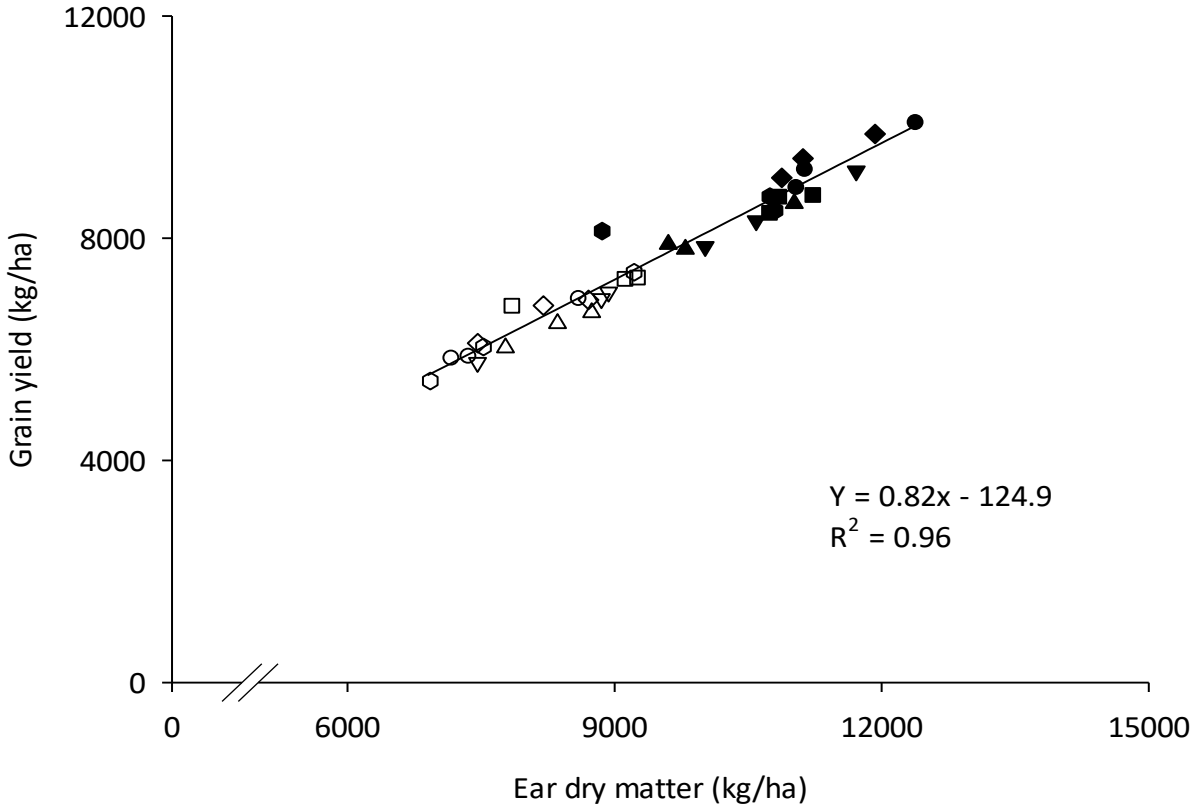

Figure 3.7: Grain biomass (kg/ha) against ear biomass (kg/ha) for six wheat genotypes (● PFR-2021, ▼ PFR-3019, ■ PFR-3026, ◆ 'Discovery', ▲'Duchess' and ⬣ 'Reliance') grown with 0 kg N/ha (open symbols) or 200 kg N/ha (closed symbols) at Lincoln, Canterbury in 2017-18 season.

3.7.5.2 Chaff (non-grain ear) biomass

Chaff biomass yield increased with increasing N fertiliser supply (P = 0.001), from 1.67±0.10 t/ha for the 0 kg N/ha to 2.04 t/ha for the 200 kg N/ha crops. However, chaff biomass yield did not differ (P=0.14) among the genotypes. The chaff biomass (Tables 3.6) was 14.0% of the total AGB (Tables 3.4).

3.7.5.3 Stem biomass at anthesis and harvest maturity

There was an interaction (P = 0.05) between N fertiliser supply and genotype (Table 3.8; Figure 3.8), at harvest maturity, because 'Duchess' produced a stem biomass of 2.53±0.05 t/ha for both N fertiliser rates. In contrast, stem biomass yield for the other five genotypes increased with increasing N fertiliser supply, from an average of 2.24±0.10 t/ha for the 0 kg N/ha crops to 2.78 t/ha when 200 kg N/ha was applied.

Stem biomass yield differed (P < 0.05) among the genotypes at anthesis and harvest maturity (Figure 3.8; Table 3.8). At both N fertiliser rates, 'Discovery' had the highest stem yield compared with 'Reliance' (Figure 3.8). Maximum stem biomass reached a peak during early milk development stage (GS73; 1,158°Cd) (Figure 3.6), when the 200 kg N/ha crops had accumulated (P < 0.001), 4.28±0.05 for 'Discovery' and 3.52 t/ha for 'Reliance'.

Table 3.8: Mean plant organ biomass yield and their proportion to total above ground biomass (% AGB) at harvest maturity for the six wheat genotypes (G) grown at low N (0 kg N/ha) or optimum N (200 kg N/ha) fertiliser supply of application at Lincoln in 2017-18.

Organ	Mean biomass yield and ranges (t/ha):		% AGB	Treatments responses[1]:		
	Nil-N	High-N		N fertiliser	G	N*G
Grain	6.50 (5.98-7.35)	8.80 (8.11-9.47)	0.55	***	*	*
Lamina	0.60 (0.50-0.70)	0.80 (0.70-1.00)	0.05	***	ns	ns
Sheath	1.00 (0.80-1.30)	1.30 (1.20-1.60)	0.08	***	**	ns
Stem	2.20 (1.90-2.60)	2.80 (2.20-3.60)	0.18	***	***	**
Chaff	1.70 (1.50-1.90)	2.00 (1.70-2.30)	0.14	**	ns	ns

[1] *P = 0.05, **P = 0.01, ***P = 0.001, ns = non-significant

At harvest maturity, the biomass partitioned into the stem + sheath was on average 26±1.20% (Table 3.8) of the total AGB. Stem + sheath partitioning was unaffected (P = 0.24) by N fertiliser supply, but differed (P < 0.001) among the genotypes. 'Discovery' had the highest stem + sheath biomass at 4.55±0.18 t/ha (30±0.80% of the total AGB), 'Duchess' was intermediate, at 3.83 t/ha (27.8%) and the other genotypes had the lowest stem + sheath biomass (3.40 t/ha; 25.0% of total AGB).

3.7.5.4 Sheath biomass yield at anthesis and harvest maturity

For sheath biomass, there was an interaction (P = 0.012) between N fertiliser supply and genotype (Figure 3.8) at anthesis. This was because 'Duchess' produced a sheath biomass of

1.86±0.05 t/ha for both N fertiliser rates. In contrast, sheath biomass for the other five genotypes increased from 1.59±0.05 t/ha for the 0 kg N/ha treatments to 1.78 t/ha when 200 kg N/ha was applied. Sheath biomass differed (P ≤ 0.001) among the genotypes at anthesis and harvest maturity (Figure 3.8; Table 3.8). 'Discovery' produced the highest sheath biomass at anthesis (1.94±0.07 t/ha) and harvest maturity (1.36±0.08 t/ha). PFR-2021 had the lowest biomass at anthesis and harvest maturity, and the other four genotypes were intermediate.

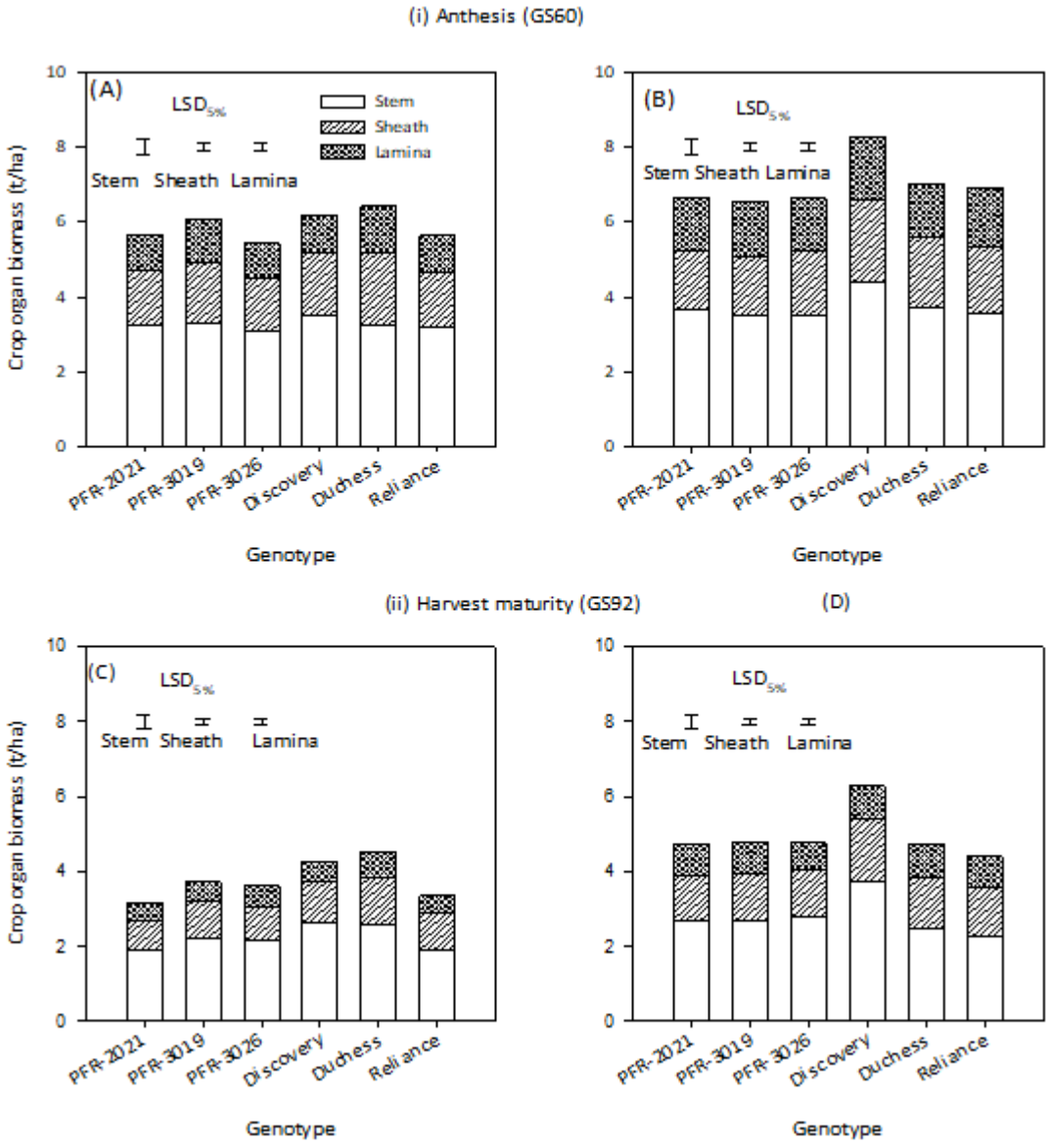

Figure 3.8: Crop organ biomass (t/ha) at (i) anthesis and (ii) harvest maturity for six wheat genotypes grown with 0 kg N/ha (A & C) or 200 kg N/ha (B & D) at Lincoln, New Zealand, during 2017-2018 season. Vertical bars are the least significant differences ($LSD_{5\%}$) for the different plant organs.

3.7.5.5 Leaf lamina biomass at anthesis and harvest maturity

Leaf lamina biomass increased ($P < 0.001$) with increasing N fertiliser supply (Table 3.8; Figure 3.8), but did not differ ($P > 0.19$) among the genotypes at anthesis or harvest maturity. At anthesis, lamina biomass was 1.10±0.04 t/ha for the 0 kg N/ha treatments and 1.50 t/ha when 200 kg N/ha was applied. At harvest maturity, lamina biomass was 0.54±0.03 t/ha for the 0 kg N/ha and 0.84 t/ha when 200 kg N/ha was applied. This was on average, 5.0% of the total AGB, and shows a ~ 50.0% remobilisation for the leaf biomass for all treatments.

Overall, total crop biomass remobilisation tended to differ ($P = 0.07$) with N fertiliser supply, but not genotype ($P = 0.48$). Total crop remobilisation was higher for the 0 kg N/ha crops at an average of 1.55±0.32 t/ha compared with 0.95 t/ha for the 200 kg N/ha crops; a decrease ($P = 0.02$) of biomass remobilisation efficiency with increasing N fertiliser supply from 22.1% to 11.2% when 200 kg N/ha was applied.

3.7.6 Crop nitrogen (N) accumulation

3.7.6.1 Nitrogen harvest index

The NHI differed ($P = 0.005$) with N fertiliser supply but the differences were small (~2.40%) and therefore an average (0.82±0.01) was used (Figure 3.9 C, D). 'Discovery' and 'Duchess' had the lowest ($P = 0.003$) NHI at 0.80±0.01 for both N fertiliser rates compared with a mean of 0.84 for the other three genotypes [PFR-3019, PFR-2021 and 'Reliance']. PFR-3026 had intermediate NHI (0.82).

3.7.6.2 Total crop N (kg/ha)

For the total AGN (Section 2.3.1) yield, there were interactions ($P < 0.01$) between N fertiliser supply and genotypes at anthesis and harvest maturity. At anthesis, 'Duchess' and PFR-3019 had higher AGN yield at 0 kg N/ha (Figure 3.9) and the lowest N yields when 200 kg N/ha was applied. At harvest maturity, AGN yield did not differ among the genotypes at 0 kg N/ha, but was higher for 'Discovery', PFR-2021 and PFR-3019 at an average 265±8.12 kg/ha, compared with 229 kg/ha for the other three genotypes, when 200 kg N/ha was applied.

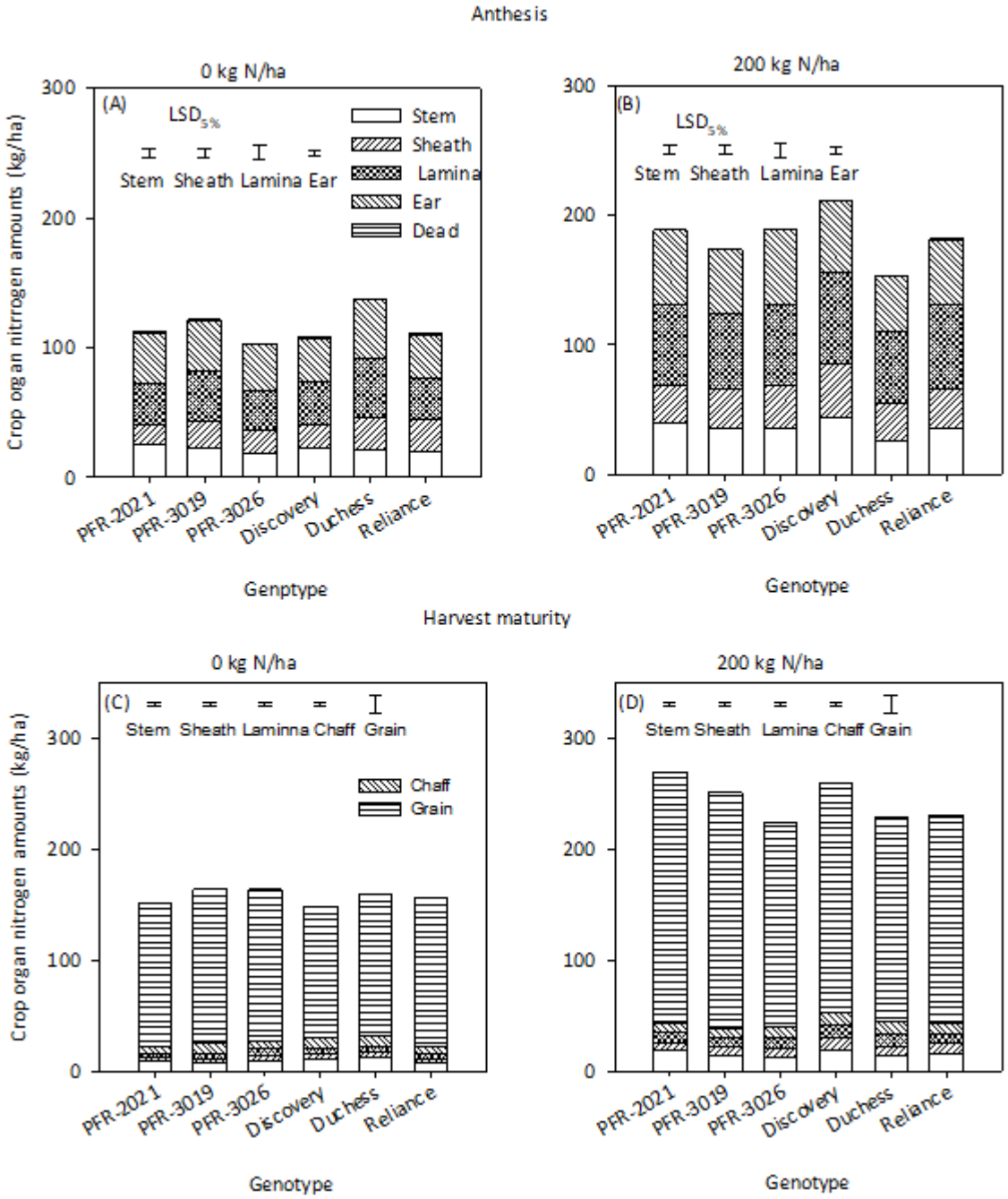

Figure 3.9: Total crop organ nitrogen (N) (kg/ha) at anthesis (A & B) and harvest maturity (C & D) for six wheat genotypes grown with 0 (A & C) or 200 kg N/ha (B & D) at Lincoln, New Zealand, during 2017-2018 season. Vertical bars are the least significant differences ($LSD_{5\%}$) for the different plant organs.

At anthesis, AGN increased ($P < 0.001$) with increasing N fertiliser supply from 116±4.7 kg/ha for the 0 kg N/ha treatments to 183 kg/ha when 200 kg N/ha was applied (Figures 3.9), a 34.0% apparent N fertiliser recovery (AFR) rate. At harvest maturity, AGN increased from 157±4.7 kg/ha for the 0 kg N/ha to 247.1 kg/ha for the 200 kg N/ha, giving an AFR rate of

45%. These figures show that crops had taken up ~74.5±3.84% of the total AGN at anthesis (Figure 3.9), and the rest post-anthesis (Section 3.8.1.1). At harvest maturity, the calculated amount of N remaining in the straw (Figure 3.9) was higher ($P < 0.001$) for the 200 kg N/ha crops at 47.0±5.0 kg/ha compared with the 26.5 kg/ha for the 0 kg N/ha treatments. However, there were no differences ($P = 0.43$) for N remaining in the straw among genotypes.

3.7.6.3 Grain N (N_g; kg/ha)

Grain N yield (N_g; kg/ha) was affected ($P = 0.02$) by the interaction between N fertiliser supply and genotype (Figure 3.9). This was because N_g yield did not differ among the genotypes at 0 kg N/ha (Figure 3.9), but was higher for 'Discovery', PFR-2021 and PFR-3019 at an average of 214±11.0 kg/ha when 200 kg N/ha was applied, compared with 185 kg/ha for the other three genotypes. The N_g yield increased ($P < 0.001$) with increasing N fertiliser supply, from an average of 130±4.5 kg/ha for the 0 kg N/ha crops to 200 kg/ha when 200 kg N/ha was applied.

3.7.6.4 Post anthesis nitrogen uptake (PANU; kg/ha)

Post-anthesis N uptake (PANU) (Section 3.6.1.3.2) increased ($P = 0.02$) with increasing N fertiliser supply (Figure 3.9) from an average of 41.1±16.2 kg/ha for the 0 kg N/ha treatments to 64.2 kg/ha when 200 kg N/ha was applied. PANU was higher ($P < 0.05$) for PFR-2021 and PFR-3019, at an average of 63.4±2.3 kg/ha compared with 47.3 kg/ha for the other four genotypes.

The signs of the partial regression coefficients (Table 3.9) show that PANU was associated negatively with pre-anthesis N status and positively with grain N yield. The implication was that PANU was dependent on ability of genotypes to translocate N to the grains. The combined variation of 36.0% in PANU was accounted for, entirely by the grain N yield (reproductive sink), as revealed by partial regression analysis. At individual N level, N_g accounted for approximately one-third and one-half of the variation in PANU, at low and optimum N fertiliser supply, respectively.

Table 3.9: Partial regression analysis for post-anthesis N accumulation on pre-anthesis N accumulation (N_{pre}) and grain N yield (N_g).

	N_{pre}			N_g			
N fertiliser (kg/ha)	*Slope*	*Intercept*	R^2	*Slope*	*Intercept*	R^2	R^2 Combined
0	-0.45	94.5	0.14	0.69	-46.3	0.36	0.50
200	-0.84	218	0.36	1.13	-163	0.48	0.84
Combined	0.001	48.5	0.003	0.42	-15.6	0.36	0.36

3.7.6.5 Grain N concentration (N_g%)

The N_g% increased ($P < 0.001$) from 2.0±0.03% for the 0 kg N/ha crops to 2.28% for the 200 kg N/ha crops (Figure 3.10). This translates to protein content of 11.4—13.1%, adequate for milling quality in New Zealand.

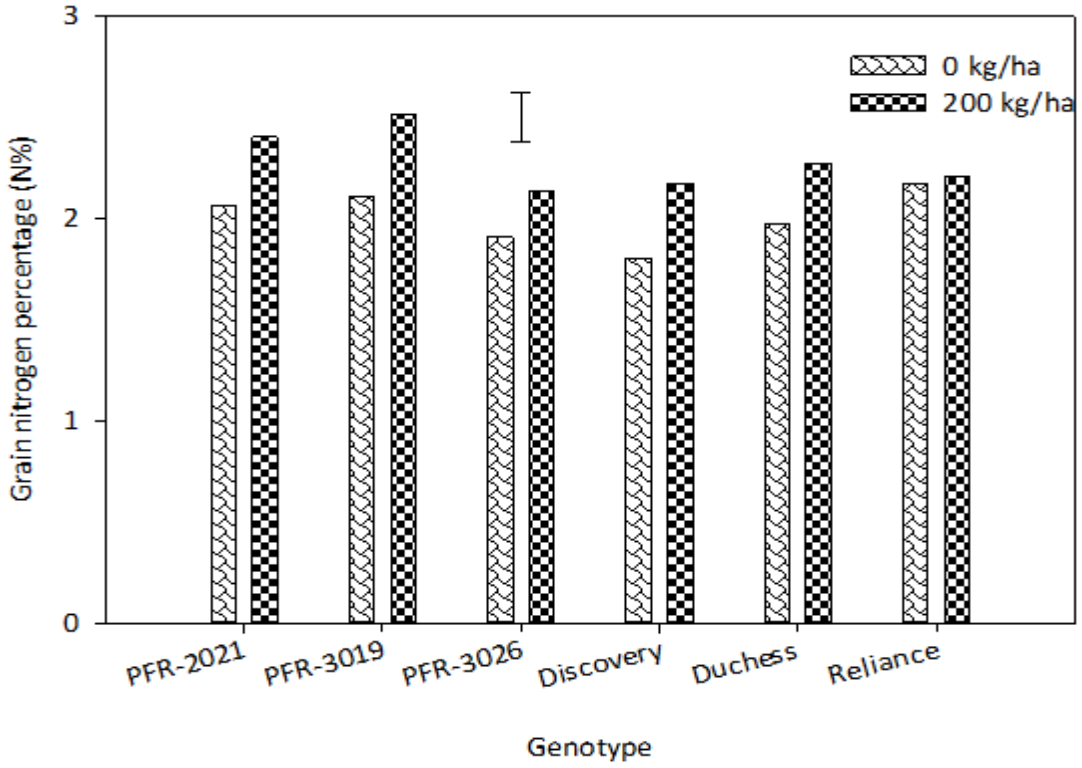

Figure 3.10: Grain N concentration (N%) for six wheat genotypes grown with 0 or 200 kg N/ha at Lincoln, Canterbury in 2017-18 season. Vertical bar is the least significant differences ($LSD_{5\%}$)

Grain N% differed ($P = 0.003$) among the genotypes (Figure 3.10). PFR-2021, PFR-3019 and 'Reliance' had the highest N_g% at ~2.24±0.08% for both N fertiliser rates. 'Discovery' and PFR-3026 had the lowest N_g% at 2.04%, while 'Duchess' was intermediate at 2.12%.

3.7.6.6 Specific leaf nitrogen (SLN; g N/m^2)

At anthesis, the SLN differed ($P < 0.001$) among the genotypes (Appendix 3.3), highest for 'Reliance' at 5.30 g N/m^2 compared with 4.0±0.23 for Discovery and PFR-2021. The SLN decreased ($P = 0.04$) with N fertiliser supply, from 4.56 g N/m^2 for the 0 kg N/ha crops to 4.27 for the optimum N fertiliser crops. As the total leaf N did not differed among the genotypes (Figure 3.9), the SLN values reported here were determined by the green leaf area (Figure 3.5), higher for the genotypes with lower green leaf area and vice-versa (Appendix 3.3).

3.7.6.7 Crop N partitioning

At harvest maturity (Figure 3.9), the partitioning of AGN was: grain (130±4.5 kg/ha and 200 kg/ha, for the 0 and 200 kg N/ha, respectively), > stems (9.40±0.98 and 15.8 kg/ha) > chaff (8.62±1.02 and 13.0 kg/ha) > sheath (4.45±0.46 and 8.82 kg/ha) > leaf lamina (4.10±0.62 and 9.50 kg/ha), for the 0 kg N/ha and 200 kg N/ha, respectively. This represented, an average of 81.7±0.66%, 6.24±0.34%, 5.36±0.41%, 3.30±0.14% and 3.40±0.22% of the total AGN, in each of the respective organs.

At anthesis, stem N yield differed ($P = 0.05$) among the genotypes (Figure 3.9), and 'Discovery' and PFR-2021 had higher stem N yields for both N fertiliser treatments. PFR-3026 had the lowest stem N yield at 0 kg N/ha and 'Duchess' had the lowest stem N yield at 200 kg N/ha. At harvest maturity, the partitioning of AGN increased with increasing N fertiliser supply, for all the organs (Figure 3.9; 3.12). Only the sheath N partitioning differed ($P = 0.04$) among the genotypes, higher for 'Discovery' and 'Duchess' (7.76±0.80 kg/ha) compared with an average of 6.10 kg/ha) for the other genotypes.

The amount of N remobilisation (NR) from each organ increased ($P < 0.001$) with increasing N fertiliser supply across the genotypes for the leaf lamina, true stem and sheath. The organ NR in decreasing order were lamina > sheath > stem; representing NRE of 52.0%, 26.4% and 21.6%, respectively.

The total crop NR (Equation 3.3) and NRE (Equation 3.5) were determined from Figure 3.9. The interaction ($P < 0.05$) between N fertiliser supply and genotypes on total crop NR was

because NR for 'Duchess' was 106±12.1 kg/ha for both N fertiliser rates (Figure 3.9). In contrast, NR for the other genotypes increased (P< 0.001) from an average of 89.0±4.9 kg/ha for the 0 kg N/ha crops to 136 kg/ha when 200 kg N/ha was applied.

3.7.6.8 Nitrogen recovery and efficiencies

Nitrogen use efficiency (NUE) and its components decreased (P < 0.001) with increasing N fertiliser supply (Table 3.10). The NUE decreased from 76.8±1.6 kg/kg for the 0 kg N/ha crops to 30.7 kg/kg when 200 kg N/ha was applied. The NupE decreased with increasing N fertiliser supply from 1.84±0.04 kg/kg to 0.87 kg/kg, while NutE decreased from 41.7±1.0 kg/kg to 35.7 kg/kg, for the 0 and 200 kg N/ha crops, respectively.

At optimum N fertiliser supply, NUE was higher (P < 0.05) for 'Discovery' and PFR-2021 at 33.1±2.73 kg/kg (Table 3.10), and was intermediate at 30.4 for PFR-3026 compared with the 29.3 kg/kg for the other three genotypes. At low N, NUE was higher for PFR-3026 (83.3 kg/kg) compared with the average of 73.6±2.73 kg/kg for PFR-2021 and 'Reliance'. The other three genotypes were intermediate at 76.1 kg/kg.

Table 3.10: Mean[1] nitrogen uptake, utilisation and use efficiency (NupE, NutE, NUE) for six wheat genotypes grown with 0 or 200 kg N/ha at Lincoln, New Zealand, during 2017-2018 season.

Genotype	Nitrogen efficiencies (kg/kg)					
	NupE		NutE		NUE	
N fertiliser (kg/ha)	0 kg	200	0	200	0 kg	200
PFR-2021	1.77_{b}	0.96_{a}	41.3_{ab}	34.4_{b}	73.0_{c}	33.0_{a}
PFR-3019	1.92_{a}	0.92_{ab}	40.2_{b}	32.4_{b}	77.3_{ab}	29.7_{ab}
PFR-3026	1.92_{a}	0.80_{b}	43.6_{ab}	38.1_{a}	83.3_{a}	30.4_{ab}
'Discovery'	1.74_{b}	0.91_{ab}	44.6_{a}	36.6_{ab}	77.6_{b}	33.2_{a}
'Duchess'	1.86_{ab}	0.80_{b}	40.4_{b}	35.4_{ab}	75.2_{bc}	28.5_{b}
'Reliance'	1.84_{ab}	0.81_{b}	40.2_{b}	37.2_{ab}	74.1_{c}	29.7_{ab}
Mean	1.84_{a}	0.87_{b}	41.7_{a}	35.7_{b}	76.8_{a}	30.7_{b}
Significance: P value ($LSD_{5\%}$)						
N fertiliser supply	< 0.001 (0.09)		< 0.001 (2.02)		< 0.001 (3.27)	
Genotype (G)	0.05 (0.14)		0.04 (3.50)		0.05 (3.27)	
N*G	0.24 (0.22)		0.67 (4.94)		0.29 (7.99)	

[1]Means with letter subscripts in common, within a column are not different at α = 0.05

'Discovery' and PFR-3026 had higher NutE at 40.8±1.7 kg/kg, compared with 36.2 kg/kg for PFR-3019 (Table 3.10). The other genotypes were intermediate at an average of 38.1 kg/kg.

NupE was higher at 1.39±0.07 kg/kg for PFR-3026, PFR-2021 and PFR-3019 compared with 1.32 kg/kg for the other three genotypes.

The NupE explained the high NUE for PFR-3026 at low N fertiliser supply and for 'Discovery' and PFR-2021 at optimum N fertiliser supply. When the relationships between NUE and its components were determined at individual N level (Table 3.10); NupE was closely related (R^2= 0.45, 0.55) to NUE at 0 and 200 kg N/ha, respectively, compared with NutE (R^2= 0.25 and 0.001) for the same N treatments.

Table 3.11: The relationship between nitrogen use efficiency (NUE) and nitrogen uptake and utilisation efficiencies (NupE, NutE), and NupE and NutE for wheat crop genotypes grown with 0 or 200 kg N/ha at Lincoln, New Zealand, during 2017-2018 season.

N Efficiency	N supply (kg/ha)	NupE	NutE
NUE	0	Y = 0.02x + 0.69; R^2 = 0.45	Y = 0.21x +26.1; R^2 = 0.25
	200	Y = 0.03x – 0.11; R^2 = 0.54	Y = -0.15x + 40.3; R^2 = 0.01
NupE	0		Y = -6.45x + 53.6; R^2 = 0.11
	200		Y = -23.0x +55.5; R^2 = 56

The relationship between NupE and NutE was negative at both N fertiliser rates (Table 3.11) and poor (R^2 = 0.11) at 0 kg N/ha and moderate (R^2 = 0.56) at 200 kg N/ha.

3.8 Discussion

The objective of this chapter was to determine the effects of genotype, N fertiliser supply and their interaction on CHI, NHI and NUE for spring wheat genotypes. Results showed that CHI, NHI and NUE differed among the genotypes. However, CHI was unaffected by N fertiliser supply, while both NHI and NUE decreased with increasing N fertiliser supply. Grain yield differed among the genotypes and increased with increasing N fertiliser supply. High grain yield was attributed to faster LAER, high maximum GLAI and longer LAD during grain filling (Section 3.7.4), and was described by mutual compensation of yield components, with either high grain density or TGW in combination with low screenings. The differences in N_g% among genotypes were attributed to the PANU and high stem N storage at anthesis. The NUE differences among the genotypes were explained mainly by NupE at both N fertiliser levels, as there was a weak relationship (R^2 = 0.25) between NUE and NutE at the low N

fertiliser supply. There was a positive relationship (R^2 = 0.54) between CHI and NHI, with a unit increase in CHI resulting in 1.5 times NHI.

3.8.1 Crop harvest index (CHI)

The differing CHI among the genotypes (Table 3.4) was a result of higher grain densities for a unit amount of biomass, coupled with stable TGW (Figure 3.4) as reported previously (Bremner & Davidson 1978; Hay 1995). All high CHI genotypes (PFR-2021, PFR-3026 and 'Reliance') had moderate to high grain densities (18,000—20,300 grains/m^2), as well as moderate TGW (39-43 g), while the genotypes with low CHI had either low grain density (~ 16,000) and high TGW (~ 50.0 g), e.g. 'Discovery', or high grain density (~ 20,110) and low TGW (~ 36.1 g), e.g. 'Duchess'. The non-response of CHI to N fertiliser supply has been reported previously (Austin et al. 1980; Ellen & Spiertz 1980; Ellen 1990; Barraclough et al. 2010; Hamnér et al. 2017) and attributed to the established weak response of CHI to environmental factors, such as plant density or fertilisation (Hay 1995) in the absence of severe stress. The current experiment was regularly irrigated, and on moderate soil fertility (Table 3.2) including soil mineral N of 85 kg/ha to 1.20 m depth. Furthermore, soil fertility was augmented by addition of basal fertilisers; therefore, crops were not stressed except for the low N fertiliser supply treatments.

Total AGB and grain yield increased with increasing N fertiliser supply (Table 3.5), while grain yield also differed among the genotypes. Differing grain yields for wheat crops with increasing N fertiliser supply was not unexpected (Foulkes et al. 1998; Barraclough et al. 2010; Gaju et al. 2011; Barraclough et al. 2014; Michel et al. 2018). Grain yield differences among the genotypes were attributed to longer LAD during the grain-filling period (Figure 3.5; 3.6). At 200 kg N/ha, the high yielding genotypes ('Discovery' and PFR-2021), attained full canopy cover ($GLAI_{crit}$) ~150°Cd earlier than the rest, and maintained $GLAI_{crit}$ for ~150°Cd after the other genotypes had opened their canopies (Section 2.3.1). This implied that they continued photosynthesising for longer, hence the high yields. The longer grain-filling period for 'Discovery' and PFR-2021 at optimum N fertiliser supply was therefore attributed to a faster LAER and longer LAD. This, coupled with a higher maximum GLAI led to delayed time to physiological maturity (Figure 3.6; Appendix 3.1). As all genotypes produced the same number of leaves, except for 'Reliance', the difference in timing of attainment of

full canopy cover was attributed to the faster LAER, and therefore leaf size. This, coupled with the high SLN of ≥ 3.90 g N/m^2, which was higher than the SLN threshold of 1.1 (0.8—1.3) g N/m^2 (Meinke et al. 1998) meant SLN did not affects photosynthetic capacity and hence reduce RUE (Sinclair & Horie 1989) among the genotypes. It was therefore concluded that the high yielding genotypes had larger leaves than the low yielding genotypes, hence more IPARi. Faster LAER and higher maximum GLAI has been achieved in maize, through breeding for more erect leaves (Duvick et al. 2004; Tollenaar & Lee 2006), which led to higher plant populations, and earlier canopy closures.

3.8.1.1 Yield components

The interaction between genotype and N fertiliser supply on grain yield (Table 3.5) highlighted the mutual compensation of the yield components (Hampton et al. 1981; Bustos et al. 2013). Grain yield for 'Discovery' was explained by the high TGW and low screenings, as it had the lowest grain density at both N fertiliser supply rates. PFR-2021 had low screenings and moderate TGW at both N fertiliser supply rates, thus the differences in grain yield were explained by the grain density, low at 0 kg N/ha compared with 200 kg N/ha (Figure 3.4). The common feature for the two high yielding genotypes ('Discovery', PFR-2021) was the low screenings, which means, they produced the number of grains that they could sustain under the prevailing conditions or they aborted excess grains earlier. The mutual compensation of the yield components was highlighted by the negative relationships between grain density and TGW (Figure 3.4), which has been reported previously (Donald 1962; Fischer et al. 1977; Scott 1981). Donald (1962) reported that if grains were larger, then because their substance is drawn from a pool of fixed carbon, the number of grains would correspondingly diminish. Fischer et al. (1977) reported that the grain density in excess of 20,000 grains/m^2 leads to drastic reductions in TGW, and grain density becomes the dominant yield determinant. In the current experiment, genotypes (PFR-3026 and 'Duchess') with the highest grain density (> 20 000 grains/m^2), had the lowest TGW (39.4, 36.1g, respectively), and 'Discovery' which had the lowest grain density (16 100±2167), had the highest TGW (50.0±1.40 g) (Figure 3.4). The importance of the trade-off between TGW and grain density is not purely speculative (Hawkesford et al. 2013; Quintero et al. 2018), as it has been reported in wheat cultivars released in Australia between 1958 and 2007 (Sadras

and Lawson 2011). This means breeders should aim for high grain density and moderate to high TGW from early biomass production, as shown for 'Discovery'.

The low yielding genotypes ('Duchess', PFR-3019 and 'Reliance'), had the highest grain density, low to moderate TGW (Figure 3.4) and highest screening at optimum N fertiliser supply. This meant the low yields were due to the low TGW and high screenings. Furthermore, 'Duchess' and 'Reliance' had lower grain yields at low N fertiliser supply. These were attributed to the lowest TGW and highest screening for 'Duchess', and the lowest grain density (similar to 'Discovery') but moderate TGW for 'Reliance'.

Comparison of low and moderate grain yielding genotypes (e.g. 'Duchess' and PFR-3026, respectively) show how different yield components determined yield. 'Duchess' and PFR-3026 had a high grain density, but 'Duchess' had lower TGW and high screenings compared with PFR-3026. The differences in grain yield were therefore due to the TGW and screenings. In contrast, at the optimum N fertiliser supply, 'Discovery' and PFR-2021 produced the same grain yield, through different mechanisms. 'Discovery' had higher TGW than PFR-2021, while PFR-2021 had higher grain density than 'Discovery'. Both had the lowest screenings compared with the other four genotypes. This meant each genotype used a different route to get to the same yield, exemplifying mutual compensation of the yield components. Furthermore, the high yielding genotypes (Table 3.5) were taller than the low yielding genotypes (Table 3.6), and had moderate to high TGW (Figure 3.4). Positive relationship between plant height and TGW has been reported previously (Johnson et al. 1966; Casebow et al. 2016). Plant height range of 0.66–0.71 m (Table 3.6) was consistent with the 0.60—0.90 cm reported for other *Rht* wheats (Austin et al. 1989; Berry et al. 2007; Gaju et al. 2011).

High grain yield with increasing TGW reported for 'Discovery' was consistent with some previous studies (Drewitt 1979; Cox et al. 1988; Calderini et al. 1995a; Sadras & Lawson 2011; Zheng et al. 2011). However, the relationship was at variance with most of the previous reports (Austin et al. 1980; Fischer 1985; Slafer et al. 1994; Sayre et al. 1997; Borrás et al. 2004; Shearman et al. 2005; Peltonen-Sainio et al. 2007; Foulkes et al. 2009a; Xiao et al. 2012), in which an increased grain density was more important in grain yield

determination. The lower grain density for 'Discovery' meant that available resources were available for the few grains, thus increased TGW. The high TGW (~ 50.0 g) and low screenings (< 1%) for 'Discovery' have been reported in recent studies (Dawson et al. 2018; Michel et al. 2018). Furthermore, the low TGW (< 44 g) and high screening for 'Duchess' (> 4.0%) have also been reported (FAR 2018; PGG-Wrightsons 2018). The negative relationship between TGW and screenings reported here was consistent with previous reports (Sharma & Anderson 2004; Nuttall et al. 2017).

In summary, present results show that grain yield differences among genotypes were attributed to faster LAER, high maximum GLAI and longer LAD, and was described by mutual compensation of yield components. For the high yielding genotypes, 'Discovery' had higher TGW than PFR-2021, while PFR-2021 had higher grain density than 'Discovery' at optimum N fertiliser supply. This meant each genotype used a different route to get to the same yield, exemplifying mutual compensation of the yield components.

3.8.2 Nitrogen harvest index (NHI)

The NHI differed among the genotypes and with N fertiliser supply (Sections 3.8.1-3.8.3). Genotypic differences for NHI to N fertiliser supply has been reported previously for 'old' wheat genotypes (Halloran & Lee 1979; Halloran 1981), as well as recent *Rht* genotypes (Gaju et al. 2011). In the current study, the overall NHI decrease of ~2.40% with increasing N fertiliser supply was small (0.83 to 0.81) and the genotypic differences, from 0.80 ('Discovery') to 0.84 (PFR-2021 & 'Reliance') were consistent with the ranges of 0.77-0.80 reported for other *Rht* genotypes (Gaju et al. 2011). The decrease in NHI with increasing N supply is associated the dilution of N by enhanced carbohydrate production in the grain. The NHI has increased with date of introduction of genotype, lower for the old genotypes at 0.25—0.52 (Halloran & Lee 1979) compared with the 0.77—0.80 (Slafer et al. 1990; Gaju et al. 2011) and 0.80—0.84 reported in the current study. The NHI differences/ ranges for the modern genotypes has become smaller, which could be attributed either to the ability of these genotypes to efficiently translocate N to the grains, or take up N post-anthesis.

Similar AGN among genotypes for the 0 kg N/ha crops (Figure 3.9), suggest that N accumulation was limited by N availability (Pask 2009), while differences among genotypes

at optimum N fertiliser supply, suggest AGN accumulation was limited by the rate and duration of N uptake and assimilation. These results are inconsistent with most published reports (Austin et al. 1977; Foulkes et al. 1998; Guarda et al. 2004), where N yield differed among genotypes at both low and optimum N fertiliser supply. However, these reported differences were either on the date of introduction of genotype (e.g. Guard et al. 2004) or height of the genotypes or a combination of time of breeding and plant height (Ortiz-Monasterio et al. 1997; Guarda et al. 2004). Present results indicate that the differences in total AGN among genotypes were driven by the stem N yield at anthesis and sheath N yield at harvest maturity. 'Discovery' and PFR-2021 had higher stem N yield at anthesis, while 'Discovery' had higher sheath N yield at harvests maturity.

The ranking of N_g% for all genotypes, except PFR-2021, followed the well-known inverse relationship between yield and N_g% (Drewitt 1979; Austin et al. 1980; Cox et al. 1985; Guarda et al. 2004; Triboi et al. 2006). The low yielding genotypes e.g. PFR-3019 and 'Reliance' had higher N_g%, thus higher grain protein content (GPC). However, since the relative variation in grain yield was greater than that in N_g%, the total amount of N in the grain was greater for the higher yielding genotypes (Section 3.8.2) (Austin et al. 1980). Conversely, the high-yielding genotypes, e.g. 'Discovery' had lower N_g% due to the well-established critical N dilution concept (Justes et al. 1994). The N_g% could also be attributed to plant height as the low N_g% genotypes were taller, and had moderate to high TGW than the high N_g% genotypes. Taller plants have more structural components, thus most of the N would be tied up in structural component of the plant. The negative relationship between N_g% and TGW has been reported in previous studies (Drewitt 1979). This was attributed to the dilution of the limited N_g by the increasing carbohydrate component of the grain.

However, PFR-2021 responded differently to the established rules on N_g% vs. grain yield or plant height. This was attributed to its higher PANU (Section 3.8.4). Previous research had shown that some genotypes are better able to accumulate higher N_g%, and hence have higher GPC than others at the same yield (Monaghan et al. 2001; Pask 2009; Bogard et al. 2010), a trait known as grain protein deviation. It is thus concluded that the main trait conferring negative departure from the overall negative relationship between yield and N_g% was either through PANU or the positive relationship between plant height and N_g%.

3.8.3 Nitrogen use efficiency and components

The decline in NUE with N fertiliser supply was consistent with previous reports (Gaju et al. 2011; Pask et al. 2012), and associated with a decline in NupE. The NUE differences among wheat genotypes was also consistent with previous reports (Cox et al. 1985; Van Sanford & MacKown 1987; Gauer et al. 1992; Fischer et al. 1993; Ortiz-Monasterio et al. 1997). The NUE differences among the genotypes (Table 3.10) were largely due to the high NupE at the respective N fertiliser supply rates, and a reflection of their high grain yield (Table 3.5). These results are consistent with previous reports (e.g. Triboi et al. 2006). The absolute values of NUE of 29.0-33.0 kg DM/kg N for the optimum N fertiliser supply crops (Table 3.10) were comparable to the 33.0-37.0 kg DM/kg N reported by Pask et al., (2012) and the 32.0 kg DM/kg N calculated from Michel at al. (2018).

The differing NUE components (NupE, NutE) among the genotypes (Table 3.10) was consistent with previous reports (Cox et al. 1986; Van Sanford & MacKown 1987; May et al. 1991; Foulkes et al. 1998). However, as most of the current breeding takes place in high yielding environments, the ability of plants to take up N is a trait that is not directly selected for (Muurinen & Peltonen-Sainio 2006). The NutE has been reported to be the trait most affected by breeding (Ortiz-Monasterio et al. 1997). This was attributed to the fact the CHI, a sub-component of NutE, has increased appreciably with breeding (Austin et al. 1980) and these improvements have contributed substantially to improved NutE (Calderini et al. 1999).

In summary, current data shows that the differences in NUE were explained mainly by NupE at low and optimum N fertiliser supply (R^2 = 0.45; 0.55, respectively). There was a weak relationship (R^2 = 0.25) between NUE and NutE at low N. These results are in agreement with Dhugga and Waines (1989), who reported that NupE was the important determinant of NUE at all N levels. However, these results are inconsistent with a large body of published reports which suggests NupE accounts for more of the genetic variation in NUE at low N than at optimum N fertiliser supply (Ortiz-Monasterio et al. 1997; Le Gouis et al. 2000; Gaju et al. 2011) or that NUE was explained approximately equally by both NupE and NutE at optimum N fertiliser supply (Foulkes et al. 1998; Muurinen et al. 2006). The current results could be attributed to the use of modern genotypes, which produced high grain yields at both N fertiliser rates (Table 3.5), similar to previous reports (Ortiz-Monasterio et al. 1997;

Foulkes et al. 1998; Brancourt-Hulmel et al. 2003). The fact that NUE among the genotypes was attributed to NupE at both N levels means that the availability and uptake of N was more important than how the plant utilised the N.

3.8.4 Conclusions

The CHI differed among the genotypes, regardless of N fertiliser supply. The high AGB and grain yields were attributed to a faster LAER, higher maximum GLAI and longer LAD. The grain yield was described by mutual compensation of yield components, with either high grain density or TGW in combination with low screenings. Grain yield for 'Discovery' was explained by high TGW, while that for PFR-2021 by the high grain density, as they both had low screenings. The NHI differed among the genotypes and decreased with increasing N fertiliser supply, however, the differences were small, and therefore mean values have been reported. The N_g% differences among genotypes were attributed to PANU and high stem N storage at anthesis. The NupE at both N levels explained the NUE differences among the genotypes.

The primary objective of this experiment was to determine the effects of genotype, N fertiliser supply and their interactions on CHI, NHI, NuHI and NUE for spring-sown wheats. Chapter 3 described the CHI, NHI and NUE. The effects of genotype and N fertiliser supply on NuHIs for other macro- and micro-nutrients will be considered in the Chapter 4.

Chapter 4: Nutrient harvest indices for six wheat genotypes grown at low and optimum N fertiliser supply.

4.1 Introduction

Chapter 3 showed that CHI, NHI and NUE differed among the six wheat genotypes, regardless of N fertiliser supply. However, NHI and NUE decreased with increasing N fertiliser supply. The implications of these results on the uptake and partitioning of other macro- (e.g. P or K) and micro-nutrients (e.g. Fe or Cu) (Sections 2.4; 2.5) are unknown. The choice of micro-nutrients (Fe, Mn, Zn and Cu) was based on their effects on crop production and their association with substantial global public health problems of micro-nutrient malnutrition (i.e. 'hidden hunger') (Loladze 2002; Myers et al. 2014). This is particularly important as most people depend on C_3 grains (known to have lower concentrations of these nutrients) for food (Myers et al. 2014). High N fertiliser use can influence the concentration of other nutrients due to an over-proportional increase in biomass production in relation to nutrient uptake (Section 2.1).

The absence of data regarding the nutritional components of modern spring wheat production systems necessitates an understanding of nutrient uptake and partitioning. The aims of this study were to understand the effects of genotype and N fertiliser supply on: (1) NuHIs, (2) temporal uptake patterns of nutrients during the life cycle of a wheat crop and (3) effect of N fertiliser supply on the concentrations of other nutrients and NuHIs for different wheat genotypes. The objective is to determine the effect of genotype, N fertiliser supply and their interaction on NuHIs and temporary nutrient uptake patterns. The null-hypotheses explored were that the: (1) NuHIs did not differ among the nutrients and genotypes, (2) nutrient accumulation did not differ with time of harvest and (3) with N fertiliser supply and that (4) N fertiliser supply did not effects the concentration of other nutrients.

4.2 Materials and Methods

The materials and methods for these crops were fully described in Section 3.2 (Experiment 1). Here additional information relevant to this chapter is described. The analyses for all the nutrients occurred at anthesis and harvest maturity growth stages.

4.2.1. Determination of mineral nutrient concentration

The harvested crops were partitioned into different organs (Section 3.3.2.1), and nutrient analyses were determined for each organ as described in the following section.

4.2.1.1 Mineral nutrient concentration

Analyses for total crop nutrient concentration were completed using the Inductively Coupled Plasma Optical Emission Spectrometry (ICP–OES; Agilent Australia Pty Ltd, Vic, Australia) (Hua et al. 2000; Nölte 2003), at Lincoln University, New Zealand. The ICP–OES is a well-established multi-element analysis technique, with a wide linear dynamic range, high analytical sensitivity, and high sample throughput. Test materials are first digested in a closed Microwave digester (CEM MARS Xpress (CEM Corporation, NC, USA)(Kingston & Jassie 1988), before analysis in the ICP–OES. Measurements of mineral nutrients were checked by the certified values of the related minerals in reference leaf and grain samples obtained from the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) as previously described (Ihnat 1994).

4.2.1.2 Plant nutrient accumulation

Total nutrient accumulated in each organ was calculated by multiplying each nutrient concentration [percentage (%) for macro- or mg/kg DM for micro-nutrients] by the plant organ biomass on a dry matter basis. The nutrient harvests indices (e.g. PHI) were determined as the ratio between grain and aboveground plant nutrient amounts at harvest maturity as shown in Equation 2.2 (Witt et al. 1999) (Section 2.3).

Nutrient accumulation at anthesis and harvest maturity was used to examine temporal uptake patterns. Specifically, nutrient remobilisation (NuR) was defined as the amount of nutrient in the crop or crop component at anthesis that was not recovered in the straw or straw components at harvest maturity (Cox et al. 1986)). This was calculated by the 'apparent remobilisation' method (Equation 3.3; Section 3.6.1.3). The post-anthesis nutrient uptake (PANuU) was defined as the amount of nutrient in the crop at harvest maturity that was not present in the crop at anthesis (Equation 3.4). Nutrient remobilisation efficiency (NuRE, %) was also determined (Equation 3.5).

4.3 Statistical analyses

Nutrient accumulation and partitioning responses were analysed using a mixed model approach, fitted with REML programme in GenStat 18th edition (Section 3.6). An estimate of the variation associated with treatment means was given by least significant difference ($LSD_{5\%}$) tests with associated d.f.. Determination of various nutrient indices, uptake patterns and remobilisation is described in Section 4.2.1. Where there was an interaction between N fertiliser supply and genotype, the ratio between interaction and main effects F-statistic values was used to determine whether to use the interaction term or main effects. Ratios greater than 10 meant the main effects were more fully explained. Unless otherwise stated, interaction are given in the text when they are significantly different ($P \leq 0.05$).

4.4 Results

4.4.1 Nutrient harvests indices (NuHI)

The NuHIs differed ($P<0.001$) among the genotypes, except ZnHI and CuHI, for which their grain accumulation (yield) did not differ among the genotypes (Table 4.1; 4.2). However, PHI was highest for the low yielding genotype, 'Reliance' (0.91) and lowest for high yielding genotype, 'Discovery' (0.81). The other genotypes were intermediate at 0.85±0.01. PFR-2021 had the highest KHI (0.26), FeHI (0.31) and MnHI (0.62), while PFR-3019 had the lowest KHI (0.19), and PFR-3026 had the lowest FeHI (0.21) and MnHI (0.50). The other genotypes were intermediate. Furthermore, 'Discovery' had the lowest PHI, KHI, and SHI, while 'Duchess' had the lowest CaHI and MgHI. The differences between other nutrients could be actual genotype attributes as they had similar biomass yields, e.g. PFR-2021 had a higher grain K yield than PFR-3019, while they had the same total K yield. Furthermore, PFR-3026 had higher Fe and Mn total yields with lower or similar grain Fe and Mn uptake compared with PFR-2021 and hence the low HIs. 'Duchess' had the lowest grain Ca yield and hence the low CaHI.

The NuHIs decreased ($P<0.001$) with increasing N fertiliser supply, except for PHI (Table 4.1) which averaged 0.85±0.01 and all micro-nutrient HIs (Table 4.2. For these NuHIs the partitioning of nutrients to the grain was similar for the low and optimum N fertiliser supply. However, KHI decreased from 0.25±0.01 for the 0 kg N/ha crops to 0.20 when 200 kg N/ha

was applied and MgHI decreased from 0.68±0.01 to 0.60 for the 0 and 200 kg N/ha treatments, respectively.

Table 4.1: Crop nutrient harvest index (NuHI; kg/kg) for macro-nutrients: phosphorus (PHI), potassium (KHI), sulphur (SHI), calcium (CaHI) and magnesium (MgHI) for six wheat genotypes grown at low or optimum nitrogen fertiliser supply (kg/ha) at Lincoln, New Zealand, during 2018-2019 season.

Genotype	Nutrient harvest index (NuHI, kg/kg)[1]									
	PHI		KHI		SHI		CaHI		MgHI	
N fertiliser	0 (kg/ha)	200	0	200	0	200	0	200	0	200
PFR-2021	0.85_{b}	0.84_{bc}	0.30_{a}	0.22_{a}	0.67_{a}	0.53_{a}	0.24_{ab}	0.19_{a}	0.69_{a}	0.58_{bc}
PFR-3019	0.86_{b}	0.88_{b}	0.22_{c}	0.16_{c}	0.64_{ab}	0.52_{ab}	0.23_{ab}	0.19_{ab}	0.58_{c}	0.56_{c}
PFR-3026	0.83_{bc}	0.84_{bc}	0.25_{b}	0.21_{ab}	0.59_{c}	0.50_{b}	0.24_{ab}	0.20_{a}	0.71_{a}	0.64_{a}
'Discovery'	0.81_{c}	0.80_{c}	0.24_{bc}	0.18_{c}	0.58_{c}	0.46_{c}	0.22_{b}	0.17_{b}	0.73_{a}	0.64_{a}
'Duchess'	0.81_{c}	0.86_{b}	0.24_{bc}	0.19_{bc}	0.59_{c}	0.48_{bc}	0.17_{c}	0.16_{bc}	0.64_{b}	0.58_{bc}
'Reliance'	0.92_{a}	0.91_{a}	0.24_{bc}	0.21_{ab}	0.66_{ab}	0.50_{b}	0.25_{a}	0.18_{a}	0.73_{a}	0.61_{ab}
Mean	0.85_{a}	0.86_{a}	0.25_{a}	0.20_{b}	0.62_{a}	0.50_{b}	0.22_{a}	0.18_{b}	0.68_{a}	0.60_{b}
Significance: P value (LSD5%)										
N fertiliser	0.46 (0.01)		<0.001 (0.01)		<0.001 (0.01)		<0.001 (0.01)		<0.001 (0.02)	
Genotype	<0.001 (0.02)		0.002 (0.02)		0.002 (0.02)		0.006 (0.02)		0.009 (0.04)	
N*G	0.54 (0.03)		0.53 (0.02)		0.50 (0.08)		0.29 (0.02)		0.70 (0.05)	

[1]Means with letter subscripts in common, within a column are not different at α = 0.05

Table 4.2: Crop nutrient harvest index (NuHI; kg/kg) for micro-nutrients: iron (FeHI), manganese (MnHI), zinc (ZnHI), and copper (CuHI) for six wheat genotypes grown at low or optimum nitrogen fertiliser supply (kg/ha) at Lincoln, New Zealand, during 2018-2019 season.

Genotype	Nutrient harvest index (NuHI, kg/kg)[1]							
	FeHI		MnHI		ZnHI		CuHI	
N fertiliser rate (kg/ha)	0	200	0	200	0	200	0	200
PFR-2021	0.28_{a}	0.29_{a}	0.62_{a}	0.63_{a}	0.63_{a}	0.58_{a}	0.18_{a}	0.19_{a}
PFR-3019	0.23_{b}	0.24_{b}	0.63_{a}	0.59_{a}	0.57_{ab}	0.64_{a}	0.17_{a}	0.15_{a}
PFR-3026	0.21_{b}	0.20_{c}	0.53_{c}	0.46_{c}	0.66_{a}	0.59_{a}	0.14_{a}	0.14_{a}
'Discovery'	0.24_{b}	0.29_{a}	0.57_{b}	0.58_{b}	0.51_{b}	0.57_{a}	0.14_{a}	0.15_{a}
'Duchess'	0.22_{b}	0.24_{b}	0.50_{c}	0.57_{ab}	0.54_{ab}	0.55_{a}	0.15_{a}	0.17_{a}
'Reliance'	0.23_{b}	0.24_{b}	0.55_{b}	0.60_{a}	0.70_{a}	0.61_{a}	0.16_{a}	0.21_{a}
Mean	0.24_{a}	0.25_{a}	0.57_{a}	0.57_{a}	0.60_{a}	0.59_{a}	0.15_{a}	0.17_{a}
Significance: P value (LSD5%)								
N fertiliser supply	0.07 (0.02)		0.57 (0.03)		0.61 (0.05)		0.30 (0.03)	
Genotype	<0.001 (0.03)		<0.001 (0.04)		0.06 (0.09)		0.36 (0.06)	
N*G	0.37 (0.04)		0.12 (0.07)		0.31 (0.13)		0.84 (0.08)	

[1]Means with letter subscripts in common, within a column are not different at α = 0.05

4.4.2 Crop nutrient concentrations (%, mg/kg DM)

Nutrient concentrations for all elements, except P, Fe and Zn were affected ($P \leq 0.02$) by developmental stage (Figure 4.1, 4.2): increased for S, Mg and Cu, decreased for K, Ca and Mn. For example, Cu increased from 4.92±0.50 mg/kg DM at anthesis to 22.7 mg/kg DM at harvest maturity, while K concentration decreased from 1.60±0.03% at anthesis to 1.0% at harvest maturity. Most nutrients concentrations were within or above the reported threshold concentrations for optimal crop growth (Reuter et al. 1997).

At anthesis, total herbage nutrient concentrations did not differ ($P \geq 0.10$) among the genotypes (Figure 4.1 A, B; 4.2 A, B). However, total herbage nutrient concentration increased with increasing N fertiliser supply for all nutrients, except Mn and Zn. For example, P% increased from 0.21±0.01% for the 0 kg N/ha crops to 0.24% when 200 kg N/ha was applied and Cu increased from 4.41±0.50 for the 0 kg N/ha to 5.43 mg/kg DM when 200 kg N/ha was applied. At anthesis, the total nutrient concentrations in whole shoots were within the ranges of 1.44±0.04—1.70% for K and 0.10—0.30% for P, S, Ca and Mg; and 80.0—100 mg for Fe, 30.0—50.0 mg for Mn and Zn, and less than 6.0 mg for Cu (Figure 4.1 A, B).

At harvest maturity, total herbage nutrient concentration for Mg, Ca, Fe, Mn and Cu differed among genotypes (Figure 4.1 C, D; 4.2 C, D). Calcium concentration was higher ($P = 0.03$) for 'Discovery' and PFR-3019, at an average of 0.17±0.01% compared with 0.13% for the other genotypes. Magnesium concentration was higher for 'Duchess' and PFR-3026 at an average of 0.12±0.01% compared with 0.10% for the other four genotypes. PFR-3026 had the highest concentrations of Fe (111 mg/kg DM), Mn (46.5 mg/kg DM) and Cu (26.6 mg/kg DM). 'Reliance' was not different to PFR-3026 for Cu concentration. These differences reflect genotypes with higher uptake for the respective nutrients because they had the same biomass yield, e.g. 'Discovery' had higher Ca and Mg uptake (Figure 4.4.) and PFR-3026 had high Fe, Mn and Cu yields (Figure 4.5). At harvest maturity nutrient concentration ranges were: 0.80—1.0% for K, and 0.10—0.30% for P, S, Ca and Mg; and 92.0±3.55 mg for Fe, and 20.0—40.0 g for Mn, Zn and Cu (Figure 4.1 C, D).

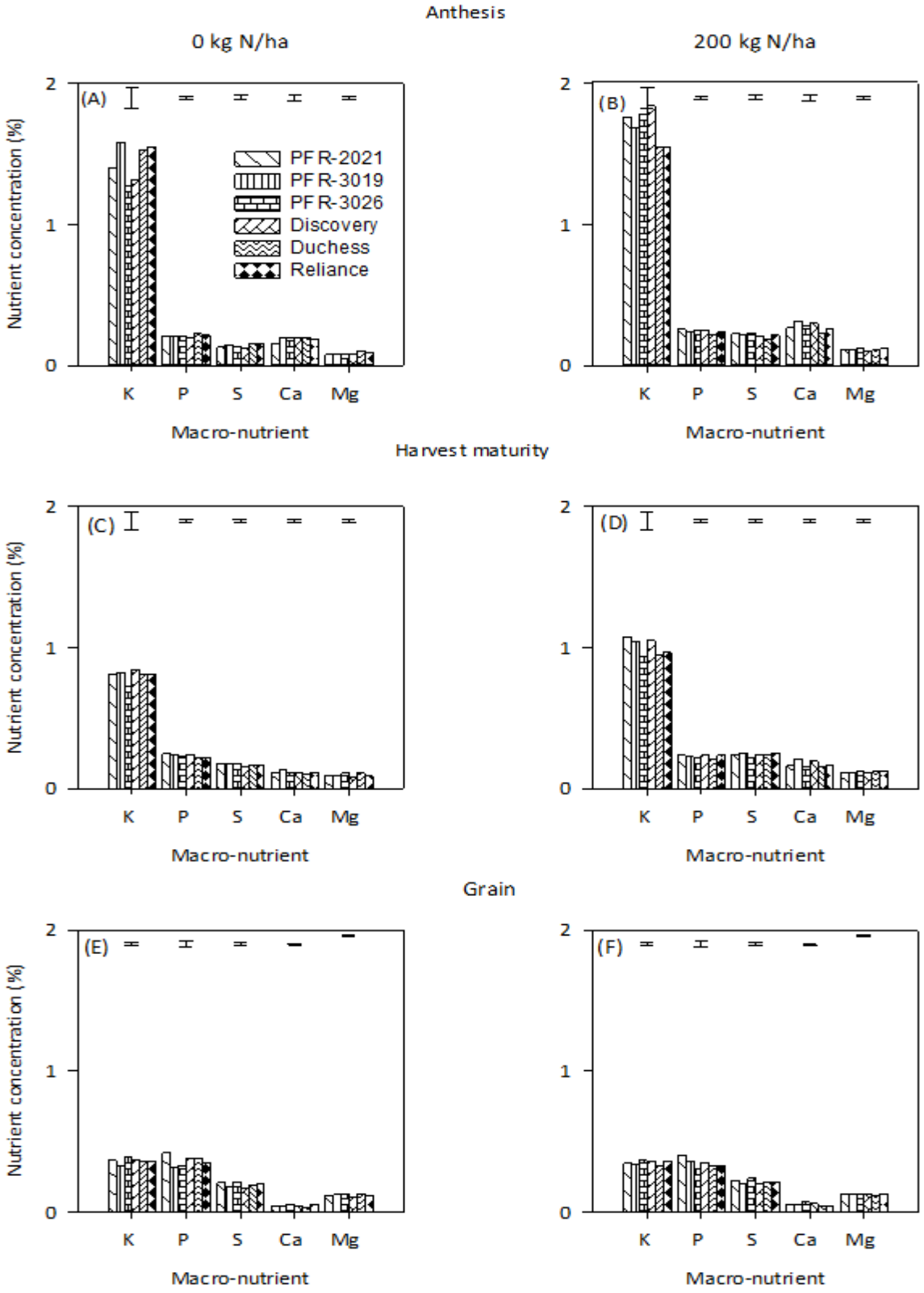

Figure 4.1: Weighted shoot (A—D) and grain (E—F) macro-nutrient concentration (%) at anthesis (A and B) and harvest maturity (C and D) for six wheat genotypes grown with 0 kg N/ha (A, C, E) or 200 kg N/ha (B, D, F) at Lincoln, Canterbury in 2017-18 season. Vertical bars are the least significant differences ($LSD_{5\%}$) for the genotypes

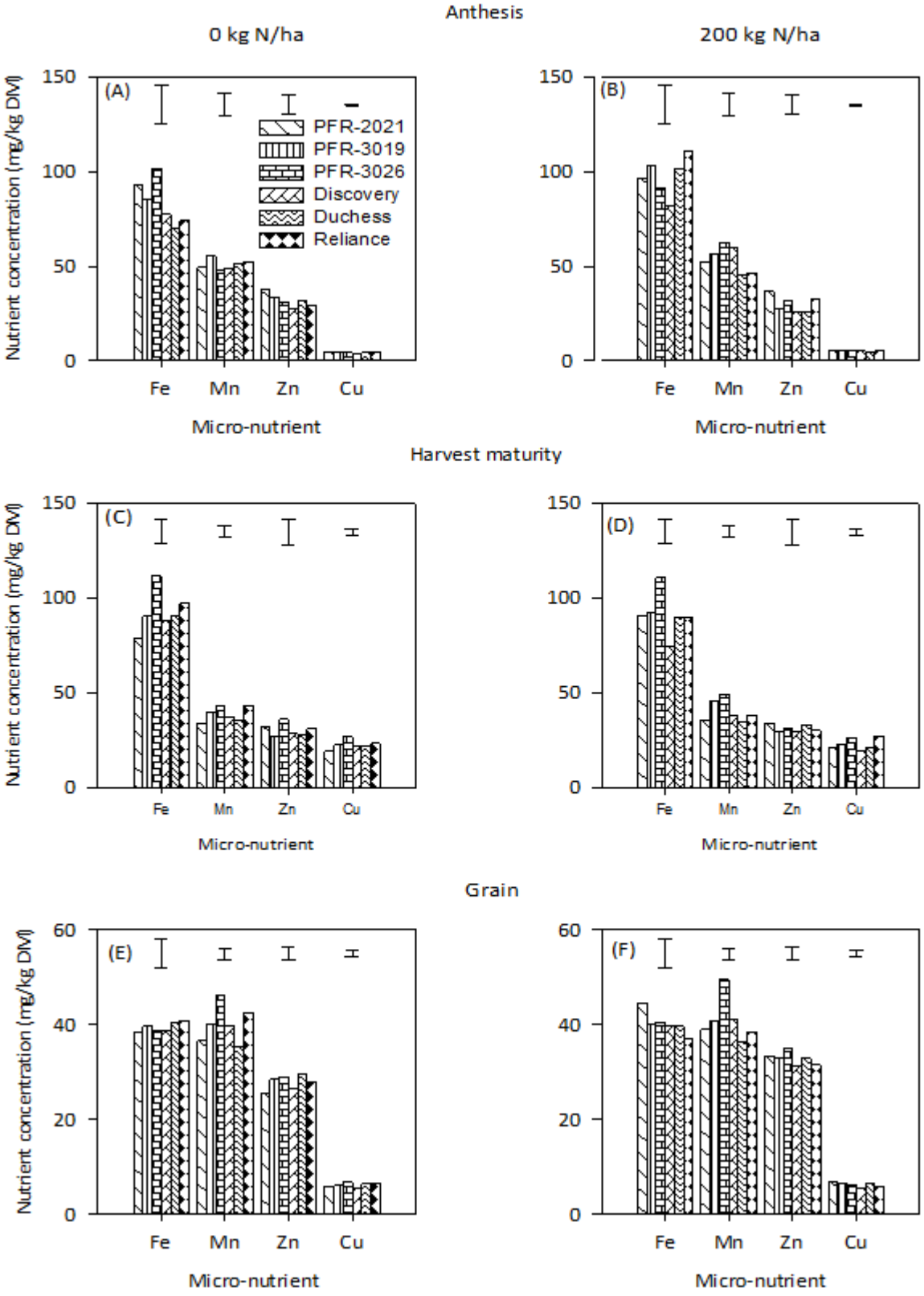

Figure 4.2: Weighted shoot (A—D) and grain (E—F) micro-nutrient concentration (mg) at anthesis (A and B) and harvest maturity (C and C) for six wheat genotypes grown with 0 kg N/ha (A, C, E) or 200 kg N/ha (B, D, F) at Lincoln, Canterbury in 2017-18 season. Vertical bars are the leaf significant differences ($LSD_{5\%}$) for the genotypes.

At harvest maturity nutrient concentration increased with increasing N fertiliser supply for all the macro-nutrients (Figure 4.1), except P. However, none of the micro-nutrient concentrations were affected ($P \geq 0.45$) by N fertiliser supply (Figure 4.2). The average micro-nutrient concentrations were 92.1±3.55 mg/kg for Fe, 22.8±0.86 mg/kg for Cu, 39.7±1.74 mg/kg for Mn and 29.1±2.20 mg/kg for Zn. As both AGB and macro-nutrient accumulation increased with increasing N fertiliser supply, the increases in macro-nutrient concentrations were due to the fact that the proportion of change in macro-nutrient accumulation was greater than that of the biomass yield increases, e.g. Ca uptake increased by ~51.0%, compared with ~31.0% for biomass yield. All micro-nutrients increased by ≤ 36.0%, which was proportional to yield increases.

4.4.2.1 Grain nutrient concentration (%; mg/kg)

Grain nutrient concentration differed ($P \leq 0.02$) among the genotypes for all nutrients except Mg (Figure 4.1 E, F), Zn and Cu (Figure 4.2 E, F). PFR-3019 had higher Ca, P and Mn and 'Discovery' had similar Ca and P concentration to PFR-3019. These genotypes had higher grain uptake amounts for these nutrients (P, Ca) and also higher post-anthesis Mn uptake (Section 4.4.3). Grain nutrient concentration response to N fertiliser supply was variable (Figure 4.1, 4.2): increased ($P < 0.001$) for Ca, S, Mg and Zn, decreased ($P = 0.01$) for P concentration and was unaffected ($P \geq 0.19$) for K, Fe, Mn and Cu. The relationship between grain N concentration (N_g%) and other mineral concentrations (Figure 4.3) was positive and close for S and Zn ($R^2 = 0.64$ and 0.90, respectively), and negative for K and P. The N_g% relationship with the other nutrients was positive but poor ($R^2 = 0.17$—0.23).

Some nutrients showed a similar pattern in grain (Figure 4.3) and vegetative stage (Appendix 4.1). Only S concentration showed a close and positive relationship to N concentration at anthesis, harvest maturity and in the grain (Appendix 4.1, Figure 4.3). In contrast, the relationship between Zn and N concentration was negative at anthesis, positive but poor ($R^2 = 0.22$) at harvest maturity and close and positive (Figure 4.3) for the grain. Some elements were positively related to N concentration at anthesis stage (Appendix 4.1) but showed negative relationship for the grains (Figure 4.3), e.g. P and K.

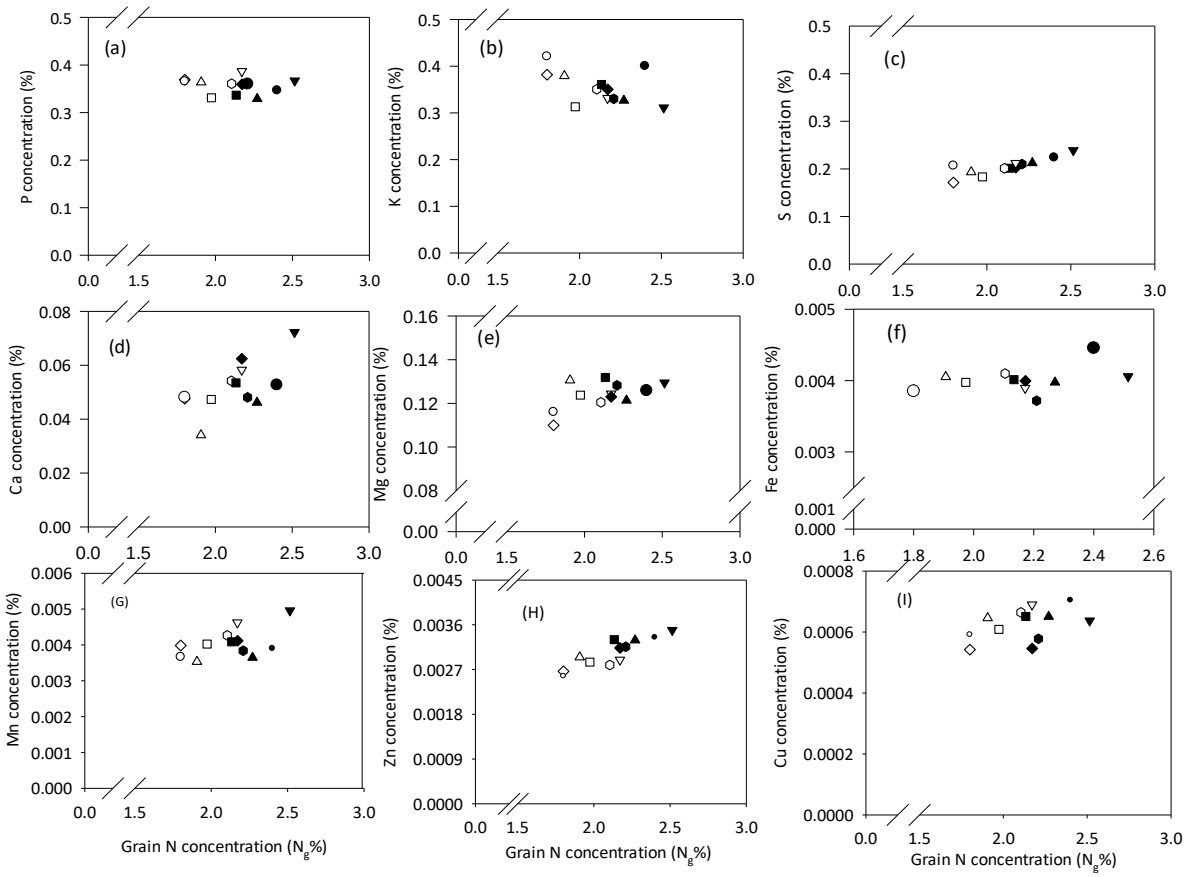

Figure 4.3: Grain nutrient against grain nitrogen concentration (N_g%) for six wheat genotypes (● PFR-2021, ▼ PFR-3019, ■ PFR-3026, ◆ 'Discovery', ▲'Duchess' and ⬣ 'Reliance') grown at low (open symbols) and optimum (closed symbols) N fertiliser supply at Lincoln, New Zealand in 2017-2018 growing season.

4.4.3 Nutrient accumulation dynamics

There were interactions ($P \leq 0.04$) between N fertiliser supply and genotype for P, K and S accumulation at anthesis (Table 4.3) but not at harvest maturity (Figure 4.4). This was because at anthesis, there were no differences between N fertiliser supply rates for 'Duchess' for the accumulation of the three elements, at an average of 17.4±0.7 kg/ha for P, 126±4.8 kg/ha for K, and 14.0±0.8 kg/ha for S. In contrast, the accumulation of all macro-nutrients increased ($P<0.001$) with increasing N fertiliser supply for the other genotypes at anthesis and harvest maturity. For example, P yield increased from 15.3±0.7 kg/ha for the 0 kg N/ha crops to 20.2 kg/ha when 200 kg N/ha was applied at anthesis (Table 4.3). At harvest maturity, P yield increased from 28.0±2.0 kg/ha for the 0 kg N/ha to 36.0 kg/ha when 200 kg N/ha was applied (Figure 4.4).

Total macro-nutrient accumulation differed (P < 0.05) among the genotypes for Ca and P at harvest maturity (Figure 4.4). 'Discovery' had the highest (P = 0.02) P yield at an average of 36.3±1.9 kg/ha compared with 29.1 kg/ha for 'Reliance'. This was because 'Discovery' had higher AGB than 'Reliance', as P concertation did not differ among the genotypes at harvest maturity (Figure 4.1). The other genotypes were intermediate at 31.7±1.9 kg P/ha. The higher (P = 0.03) Ca yields for 'Discovery' and PFR-3019 (24.4±0.3 kg/ha) compared with 18.7 kg/ha for the other four genotypes, was because 'Discovery' and PFR-3019 had higher Ca concentration at 0.17±0.01% compared with the 0.13% for the other genotypes.

Table 4.3: Macro-nutrient herbage nutrient accumulation (kg/ha) at anthesis for six wheat genotypes grown at low or optimum nitrogen (N) fertiliser supply (kg N/ha) at Lincoln, New Zealand in 2017-18 season.

Genotype	Nutrient accumulation (kg/ha)[1]									
	Phosphorus		Potassium		Sulphur		Calcium		Magnesium	
N fertiliser	0	200	0	200	0	200	0	200	0	200
PFR-2021	15.6_{ab}	20.8_{a}	105_{ab}	145_{bc}	9.60_{a}	18.2_{ab}	11.5_{a}	21.0_{a}	5.80_{ab}	9.0_{a}
PFR-3019	15.8_{ab}	18.4_{a}	120_{a}	134_{bc}	11.0_{a}	16.7_{ab}	15.0_{a}	23.8_{a}	6.20_{ab}	8.60_{a}
PFR-3026	13.6_{b}	20.5_{a}	86.5_{b}	149_{b}	9.0_{a}	19.0_{a}	12.5_{a}	22.8_{a}	5.70_{ab}	10.0_{a}
'Discovery'	14.8_{ab}	24.1_{a}	98.2_{ab}	184_{a}	9.20_{a}	20.4_{a}	13.8_{a}	28.8_{a}	5.60_{ab}	10.5_{a}
'Duchess'	17.5_{a}	17.6_{a}	119_{a}	128_{c}	12.2_{a}	14.9_{b}	15.1_{a}	17.5_{a}	8.00_{a}	8.60_{a}
'Reliance'	14.8_{ab}	20.0_{a}	108_{ab}	132_{bc}	10.2_{a}	17.5_{ab}	12.2_{a}	21.6_{a}	6.20_{ab}	9.70_{a}
Mean	15.3_{b}	20.2_{a}	106_{b}	145_{a}	10.2_{b}	17.8_{a}	13.4_{b}	22.6_{a}	6.30_{b}	9.40_{a}
Significance: P value (LSD5%)										
N fertiliser	<0.001 (1.35)		<0.001 (9.83)		<0.001 (1.55)		<0.001 (2.70)		<0.001 (0.90)	
Genotype	0.316 (2.35)		0.122 (17.0)		0.96 (2.68)		0.208 (4.67)		0.793 (1.56)	
N*G	0.01 (3.32)		<0.001 (24.1)		0.04 (3.79)		0.199 (6.61)		0.124 (2.20)	

[1]Means with letter subscripts in common, within a column are not different at α = 0.05

The temporal nutrient accumulation pattern differed among nutrients but was unaffected by N fertilisation supply. Potassium and Ca were taken up early in the growing season, as reflected by their proportion in the herbage at anthesis (100%, 92.0%, respectively) (Table 4.3) compared with harvest maturity (Figure 4.4). The implication was there was little or no uptake of K and Ca after anthesis. The proportion of total macro-nutrient at anthesis (Table 4.3) compared with harvest maturity (Figure 4.4), was on average 57.0±0.03% for P, 100±0.1% for K, 48.0±0.03% for S, 92.0±0.1% for Ca and 51.0±0.04% for Mg. Proportion for N was 74.5±3.84% (Section 3.8.2). None of these were affected (P ≥ 0.27) by N fertiliser supply or differed (P ≥ 0.46) among the genotypes.

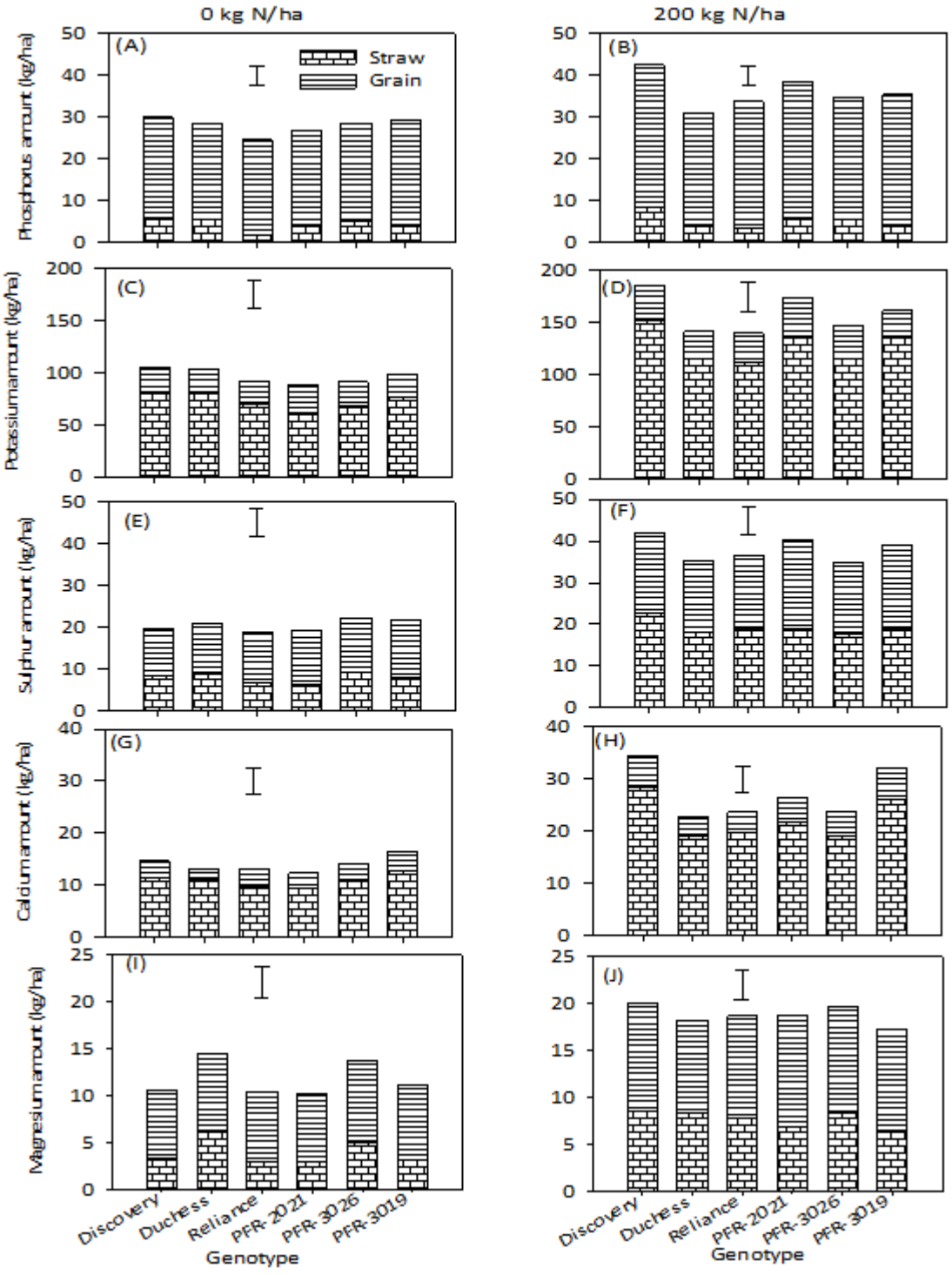

Figure 4.4: Total macro-nutrient (straw + grain) accumulation (kg/ha) at harvest maturity for six wheat genotypes grown with 0 kg N/ha (A, C, E, G, I) or 200 kg N/ha (B, D, F, H, J) fertiliser at Lincoln, New Zealand in 2017-18 season. Vertical bars are the least significant differences ($LSD_{5\%}$) for the genotypes within each N fertiliser supply rate.

At anthesis, there was an interaction (P = 0.03) between N fertiliser supply and genotype for Mn (Table 4.4), because there was no difference between N fertiliser supply rates except for

'Discovery' and PFR-3026. Micro-nutrient accumulation did not differ (P ≥ 0.21) among the genotypes at anthesis, but increased (P < 0.01) with increasing N fertiliser supply for all micro-nutrients except for Zn.

At harvest maturity (Figure 4.5), total herbage micro-nutrient accumulation differed (P ≤ 0.01) among the genotypes for all nutrients, except for Zn. In all cases, PFR-3026 accumulated more nutrients (Fe, Mn and Cu) compared with the other genotypes. This was because PFR-3026 had higher post-anthesis uptake for these three elements compared with the other genotypes (Table 4.4; Figure 4.5). For example, PFR-3026 accumulated 656 g/ha of Mn, compared with an average of 526±25.7 g/ha for the other genotypes. All micro-nutrients increased with increasing N fertiliser supply at harvest maturity (Figure 4.5). For example, Mn increased (P < 0.001) from 464 g/ha for the 0 kg N/ha crops to 632 g/ha when 200 kg N/ha was applied.

Table 4.4: Micro-nutrient herbage accumulation (g/ha) at anthesis for six wheat genotypes grown at low or optimum nitrogen (N) fertiliser supply (kg/ha) at Lincoln, New Zealand in 2017-18 season.

Genotype	Nutrient accumulation (g/ha)[1]							
	Iron		Manganese		Zinc		Copper	
N fertiliser (kg/ha)	0	200	0	200	0	200	0	200
PFR-2021	693_{a}	726_{ab}	351_{a}	427_{ab}	253_{a}	291_{a}	35.0_{a}	47.5_{ab}
PFR-3019	640_{ab}	782_{ab}	413_{a}	387_{b}	253_{a}	213_{bc}	33.8_{ab}	44.3_{ab}
PFR-3026	629_{ab}	695_{b}	354_{a}	517_{ab}	210_{b}	216_{bc}	28.6_{b}	45.8_{b}
'Discovery'	565_{b}	693_{b}	363_{a}	586_{a}	205_{bc}	250_{b}	27.0_{b}	49.9_{a}
'Duchess'	506_{bc}	811_{a}	403_{a}	363_{b}	243_{ab}	204_{bc}	35.4_{a}	40.3_{c}
'Reliance'	638_{ab}	790_{ab}	362_{a}	395_{ab}	202_{c}	193_{c}	31.9_{b}	44.6_{bc}
Mean	612_{b}	750_{a}	374_{b}	446_{a}	228_{a}	228_{a}	31.9_{b}	45.4_{a}
Significance: P value (LSD5%)								
N fertiliser	0.01 (107)		0.01 (51.1)		0.99 (36.7)		<0.001 (3.33)	
Genotype	0.90 (186)		0.21 (88.5)		0.29 (63.6)		0.76 (5.76)	
N*G	0.73 (263)		0.03 (125)		0.63 (90.0)		0.07 (8.15)	

[1]Means with letter subscripts in common, within a column are not different at α = 0.05

The proportion of total micro-nutrient at anthesis (Table 4.4), compared with harvest maturity (Figure 4.5), was on average 56.0±0.05% for Fe, 76.0±0.05% for Mn, 56.0±0.04% for Zn, and 13.0±0.01% for Cu. The proportions decreased with increasing N fertiliser supply for Zn and Mn, e.g. from 82.0% for the 0 kg N/ha crops to 71.0% when 200 kg N/ha was applied for Mn. Proportions differed among the genotypes for Cu only; highest for PFR-2021

at 16.0% compared with 10.0±0.01% for 'Reliance' and PFR-3026. The other genotypes were intermediate, at 13.0±0.01%.

4.4.3.1 Nutrient accumulation in the grain

There was an interaction ($P = 0.003$) between N fertiliser supply and genotype on Fe accumulation only; as the total accumulated Fe did not differ with N fertiliser supply for 'Duchess', PFR-3026 and 'Reliance', at 257±22.4, 282±22.4 and 249±22.4 g/ha, respectively. In contrast, Fe accumulation for the other genotypes increased ($P < 0.001$) with increasing N fertiliser supply from 250±13.0 g/ha for the 0 kg N/ha crops to 402±13.0 g/ha when 200 kg N/ha was applied. Overall, grain nutrient accumulation differed ($P < 0.05$) among the genotypes, for Ca, P and Mn (Figure 4.4; 4.5). PFR-3019 had the highest grain Ca, P and Mn amounts, at 4.97±0.44 kg/ha, 28.2±1.4 kg/ha and 360±14.5 g/ha, respectively, and 'Duchess' had the lowest Ca, P and Mn amounts (2.96 kg/ha, 25.0 kg/ha, and 260 g/ha, respectively). The differences in total accumulation for these nutrients were attributed to high nutrient concentrations reported in Section 4.4.2.1, as there were no AGB differences among the genotypes except for 'Discovery' and 'Reliance'. Nutrient accumulation increased ($P < 0.05$) with increasing N fertiliser supply for all nutrients. For example, P increased ($P < 0.001$) from 23.6±0.80 kg/ha for the 0 kg N/ha to 30.7 kg/ha when 200 kg N/ha was applied and Cu increased from 40.8±4.43 g/ha for the 0 kg N/ha to 62.0 g/ha when 200 kg N/ha was applied.

4.4.4 Nitrogen to nutrient ratios (e.g. N: P or N: Mn)

The total plant N: nutrient ratios differed ($P < 0.001$) with developmental stage for all nutrients except for N: Fe (160±26.0) and N: Zn (477±89.0) (Figure 4.6; 4.6). The N: nutrient ratios increased with developmental stage, and were higher at harvest maturity for N: K, N: Ca and N: Mn but decreased for N: P, N: Mg, N: S and N: Cu. For example, N: K increased from 1.20±0.10 at anthesis to 1.62 at harvest maturity and N: Mn increased ($P < 0.001$) from an average of 259±13.0 to 372 at anthesis and harvest maturity, respectively. In contrast, N: P decreased ($P < 0.001$) from 8.29±0.74 at anthesis, to 6.28 at harvest maturity. The N: Cu ratio decreased from 2680±59.0 at anthesis to 650 at harvest maturity. These responses were associated with the nutrient accumulation dynamics; as those elements that were taken up earlier in the season (e.g. K or Ca) showed higher ratios at harvest maturity because of the continued N uptake with developmental stage. In contrast, those element

which were mostly taken up later in the season (e.g. P or Cu), at more than the rate of N uptake, had lower ratios.

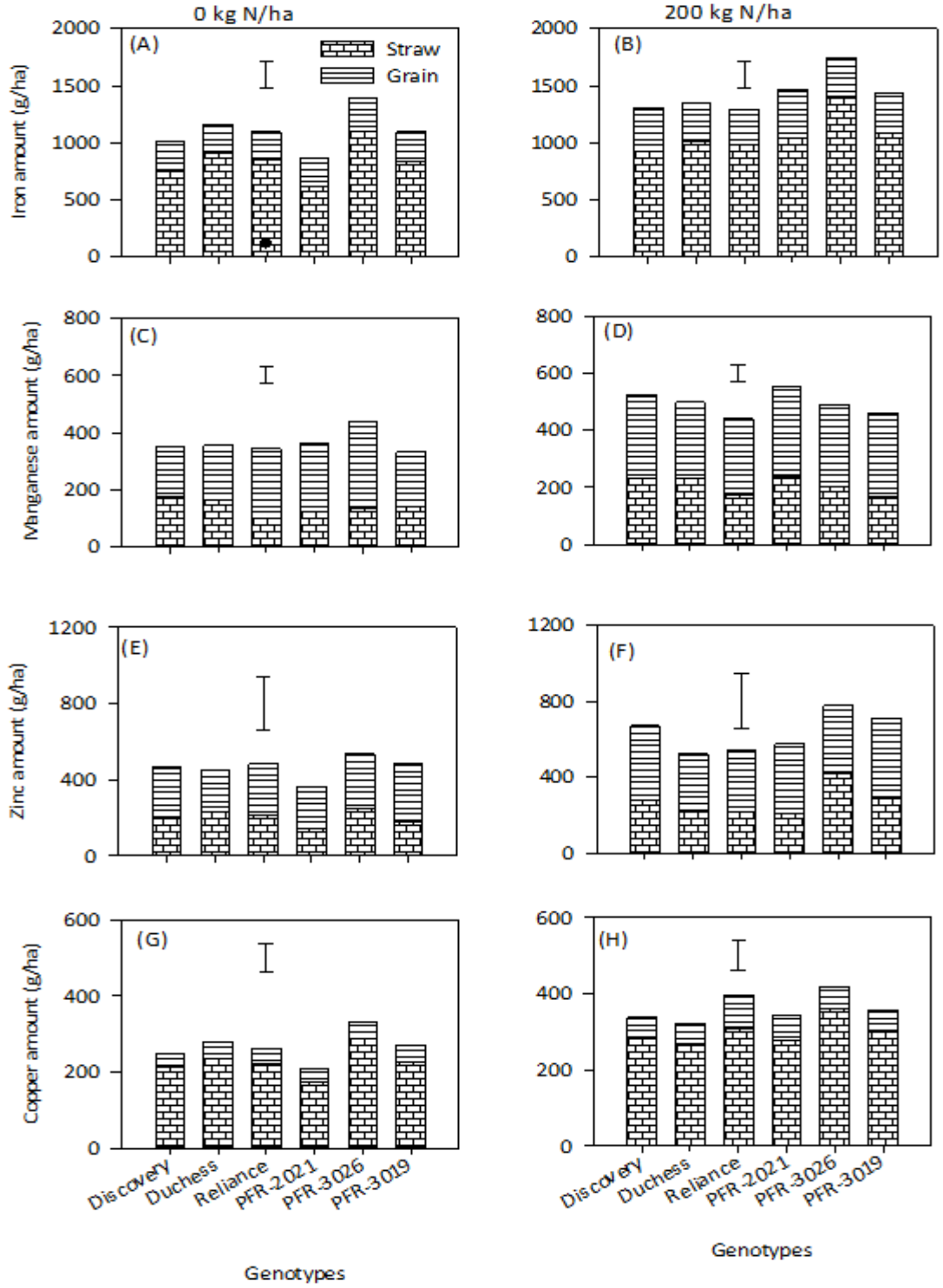

Figure 4.5: Total micro-nutrient (straw + grain) accumulation (g/ha) at harvest maturity for six wheat genotypes grown with 0 kg N/ha (A, C, E, G) or 200 kg N/ha (B, D, F, H) fertiliser at Lincoln, New Zealand in 2017-18 season. Vertical bars are the least significant differences ($LSD_{5\%}$) for the genotypes within each N fertiliser supply rate.

There was an interaction between N fertiliser supply and genotypes for N: Cu ratio at harvest maturity only (Figure 4.7). This was because N: Cu ratio was unaffected by N fertiliser supply for PFR-2021, PFR-3026 and 'Reliance', at an average of 761±17.7, 537 and 597, respectively. In contrast, N: Cu increased from 592±17.7 for the 0 kg N/ha crops to 748 when 200 kg N/ha was applied for the other genotypes. At anthesis, N: Ca and N: Cu ratios differed ($P \leq 0.05$) among the genotypes (Figure 4.6, 4.7). PFR-3019 and 'Discovery' had lower ($P = 0.04$) N: Ca ratio at an average of 7.75±0.61 compared with 8.95 for the other genotypes, while 'Discovery' had higher N: Cu ratio at 3,150±172 compared with 2,584 for the other genotypes. At harvest maturity, N: mineral ratios differed among genotypes for all nutrients except N: P and N: S. For example, PFR-3019 and 'Discovery' had a lower ($P = 0.02$) N: Ca ratio of 9.10±0.64 compared with 10.4 for the other genotypes and 'Duchess' and PFR-3026 had lower ($P = 0.003$) N: Mg ratio at 11.8±0.63, compared with 14.4 for the other genotypes. PFR-2021 had the highest N: Fe and N: Mn ratios, while 'Discovery' had higher N: Fe and N: Cu ratios. PFR-3026 had the lowest N: Fe, N: Mn and N: Cu ratios. The higher ratios for PFR-2021 and 'Discovery' were attributed to the high N uptake for the optimum N fertiliser supply (Section 3.8.1) and low Fe, Mn and Cu uptake (Section 4.4.3), while low ratios for PFR-3026 was due to its low N uptake (Section 3.8.1) and the high Fe, Mn and Cu uptake (Section 4.4.3).

At anthesis, N: nutrient ratios (Figure 4.6) were affected ($P < 0.001$) by N fertiliser supply for all elements, except for N: Ca ($P = 0.14$). For example, N: P increased from 7.60±0.23 for the 0 kg N/ha crops to 9.20 when 200 kg N/ha was applied, while N: Zn increased from 523±66.3 for the 0 kg N/ha to 784 when 200 kg N/ha was applied. However, N: S decreased from 11.4±0.17 at 0 kg N/ha to 10.4 when 200 kg/ha was applied. At harvest maturity, N: nutrient ratios (Figure 4.6; 4.7) increased with increasing N fertiliser supply for N: P, N: Cu, N: Fe, and N: Mn, decreased for N: S and N: Ca and were unaffected for N: K, N: Mg and N: Zn.

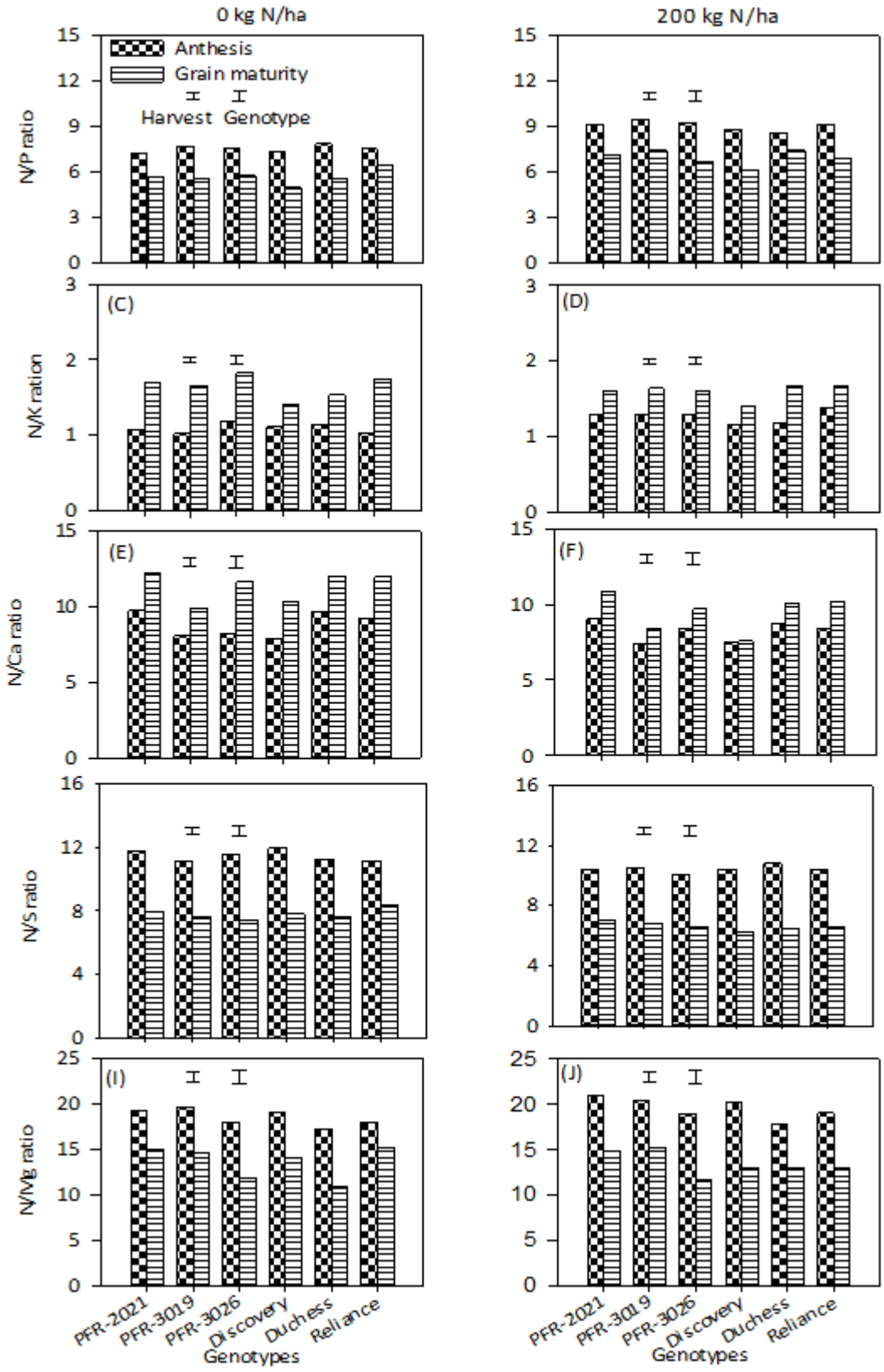

Figure 4.6: Plant nitrogen/macro-nutrient ratios at anthesis and harvest maturity for six wheat genotypes grown with 0 kg N/ha (A, C, E, G, I)) or 200 kg N/ha (B, D, F, H, J) fertiliser supply at Lincoln, New Zealand in 2017-18 season. Vertical bars are the least significant differences ($LSD_{5\%}$) for the: Harvests, N fertiliser supply and Genotypes.

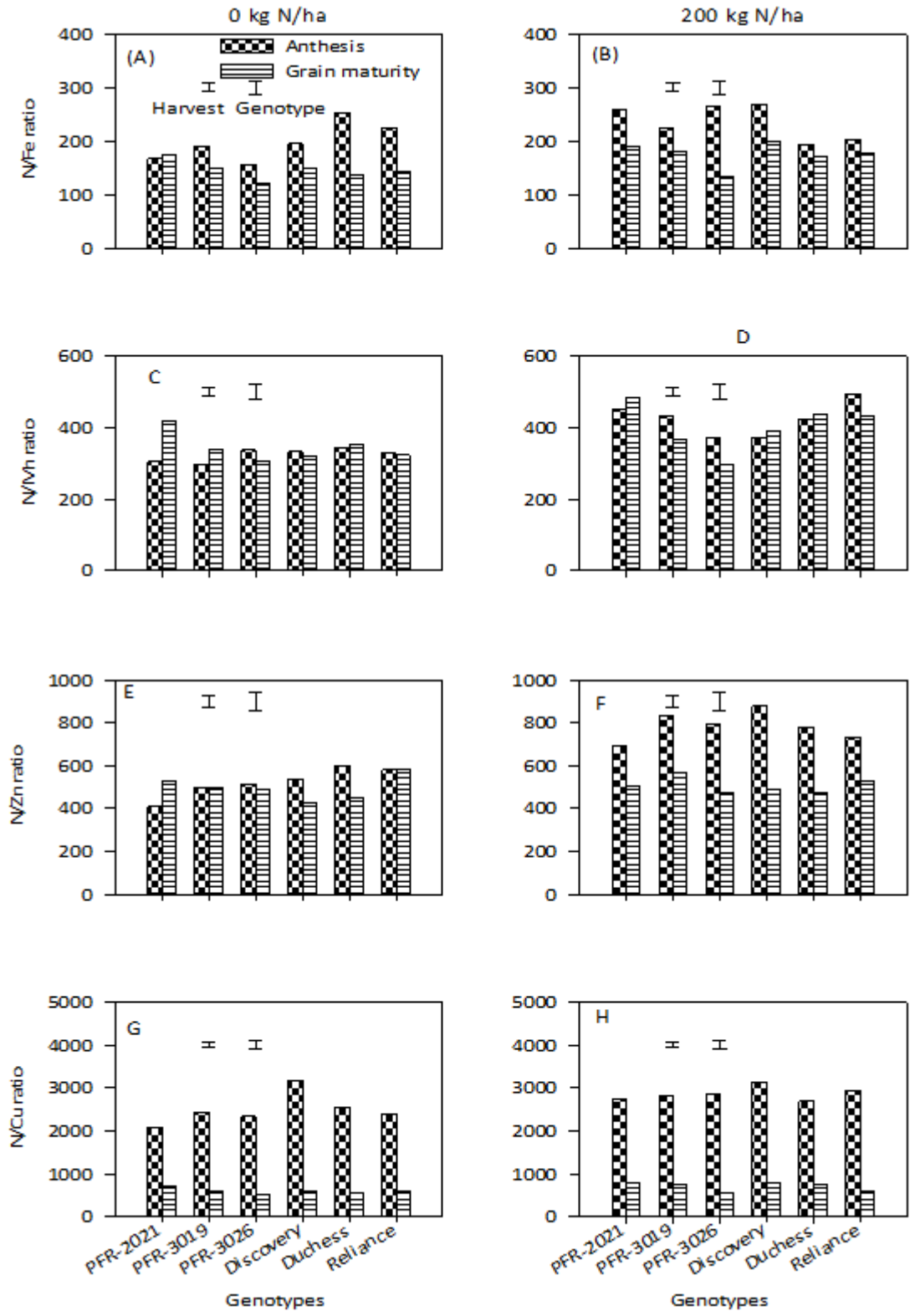

Figure 4.7: Plant nitrogen/micro-nutrient ratios at anthesis and harvest maturity for six wheat genotypes grown with 0 kg N/ha (A, C, E, G) or 200 kg N/ha (B, D, F, H) fertiliser supply at Lincoln, New Zealand in 2017-18 season. Vertical bars are the least significant differences ($LSD_{5\%}$) for the: Harvests, N fertiliser supply and Genotypes.

For the grain component, the N: K, N: Ca, and N: Mn ratios differed (P<0.05) among the genotypes (Appendix 4.2). The N: K ratio for PFR-3019 was higher (P<0.05) at 7.34±0.51 compared with 5.94 for the other genotypes, because it had a lower K yield than the other genotypes. 'Duchess' had a higher (P<0.05) N: Ca (54.3±3.80) ratio compared with the other genotypes, because it had a lower Ca yield than all other genotypes. The grain N: nutrient ratios increased (P<0.001) with increasing N fertiliser supply for all nutrients, except for N: Ca, N: Zn and N: Cu ratios. For example, the N: P ratio increased (P<0.001) from 5.54±0.17 for the 0 kg N/ha treatments to 6.55 when 200 kg N/ha was applied, and N: K increased (P<0.001) from 5.61±0.30 to 6.73, and N: Mn from 506±42.0 to 566 for the 0 and 200 kg N/ha treatments, respectively.

4.4.5 Nutrient remobilisation (NuR) and post-anthesis nutrient uptake (PANuU)

Nutrient remobilisation (NuR) and its efficiency (NuRE) were determined from data in Section 4.4.3. The NuRE decreased (P < 0.01) with increasing N fertiliser supply for all nutrients, except for P, Fe and Mn. For example, NuRE for K decreased from 30.7±5.1% for the 0 kg N/ha treatments to 10.6% when 200 kg N/ha was applied, and NuRE for Mn decreased from 32.0±8.5% to 0% for the 0 and 200 kg N/ha treatments, respectively. For Fe and Cu, the total amount of nutrient in the straw at harvest maturity (Figure 4.5) was greater than the amount accumulated at anthesis (Table 4.3). The implication was that these crops took up more Fe and Cu post-anthesis, and retained them in the straw, and hence the negative remobilisation. The NuREs differed (P > 0.02) among the genotypes for P and Mn. The NuRE for P was highest (P = 0.005) for 'Reliance' at 85.0±5.0%, intermediate for PFR-3019 (75.0%), and lowest for the other four genotypes at 63±50%. The NuRE of 22.7±8.2% for Mn was lower ((P = 0.02) for PFR-3026 compared with the 44.0% for the other genotypes.

Post-anthesis nutrient uptake (PANuU) was determined from data in Tables 4.3 and 4.4and Figure 4.4 and 4.5. PANuU differed among the genotypes for Fe, Mn and Cu only. PFR-3026 had higher (P ≤ 0.05) PANuU values for these elements, compared with the other genotypes. PANuU increased (P < 0.05) with increasing N fertiliser supply for all the nutrients, except Ca. Overall, PANuU were in the ranges of 1—10% for K and Ca; 20—30% for Mn, 40—60% for P, S, Mg, Zn and Fe; and >80% for Cu. This was a reflection of the temporal nutrient uptake patterns (Section 4.4.3).

4.6 Discussion

The objectives of this study were to determine the effect of genotype, N fertiliser supply and their interaction on NuHIs and temporary nutrient uptake patterns. The null-hypotheses explored were that: (1) the NuHIs did not differ among the nutrients and genotypes, (2) nutrient accumulation did not differ with time of harvest and (3) with N fertiliser supply and that (4) N fertiliser supply did not effects the concentration of other nutrients. Results show that the genotypes used in this study did not differ in AGB yield (Table 3.5) except for the highest yielding, 'Discovery' and lowest yielding 'Reliance'. Thus, genotypic differences reported here were attributed to the genetic difference in uptake of nutrients and were not caused by differences in biomass accumulation. The NuHIs differed widely among nutrients, genotypes (except for ZnHI and CuHI) and with increasing N fertiliser supply (except for PHI and all micro-nutrient HIs). In the herbage, a higher concentration of N resulted in increased concentrations of the macro-nutrients, but no response for the micro-nutrients. Implication was that crops were accumulating micro-nutrients at the same rate as AGB accumulation for both N fertiliser supply rates. Nutrient accumulation was split evenly pre- and post-anthesis, except for Ca, K and Mn that were accumulated earlier in the growing season, and Cu that was accumulated later during growth. The relationship between N_g% and S and Zn concentrations were close and positive (R^2 = 0.64; 0.90, respectively), while that to K concentration was negative.

4.6.1 Nutrient harvest indices

Contrary to the first hypothesis, results show that the overall NuHI values differed (Table 4.1, 4.2), ranging from low (≤0.30) for CaHI, CuHI, FeHI and KHI, moderate (0.30—0.60) for MnHI, SHI and ZnHI and high (>0.60) for MgHI, NHI and PHI. The NuHIs were a reflection of individual nutrient phloem mobility (Loneragan et al. 1976; Reuter & Robinson 1997). The NuHIs were lower for the phloem immobile nutrients such as Ca and Fe compared with phloem mobile nutrients such as Mg, N and P (Table 4.1; 4.2), and intermediate for those of variable phloem mobility e.g. S and Zn. Notable exceptions where the low KHI and moderate MnHI, as K is highly phloem-mobile and Mn is phloem-immobile. The low KHI is consistent with K being bound in structural lignin and cellulose in the stems in wheat (Gregory et al. 1979; Waldren & Flowerday 1979), which explains the high proportion in the straw

component (Figure 4.4). The low KHI has been reported in other cereals, such as maize (Karlen et al. 1988) and grain sorghum (Hocking 1993). Furthermore, the translocation of K into the grain may be affected by the foliage K losses through leaching (Tukey 1970), with differences also expected between studies due to variable rainfall and irrigation patterns. The moderate to high MnHI contradicts the idea that Mn is phloem immobile (Reuter & Robinson 1997), and accumulates in old leaves. However, there are reports of Mn being present in phloem sap at high concentrations relative to tissue requirements (Shelp 1988) and that Mn may be rapidly translocated from the stems and petioles of some species, e.g. *Lupinus angustifolius* during seed development (Hannam et al. 1985). It is unclear if the same processes occur in wheat crops, but these results suggests so.

The NuHI results reported here are consistent with those reported for winter wheat (Hamnér et al. 2017), except for FeHI and CuHI for which they reported higher values (0.63 and 0.59; respectively) compared with ≤0.30 in the current study. For Fe, a combination of a higher post—anthesis uptake, and being immobile within plants (Reuter & Robinson 1997), means less translocation to the grain and hence the low FeHI reported here. For Cu, the variable mobility (*Loc. cit*), and high accumulation in the straw (Figure 4.5) meant a low CuHI. The NuHIs for micro-nutrients are variable due to the low crop removal rates compared with total soil content (Kirchmann et al. 2013) and the natural soil variation. The NuHIs results in our study are consistent with those reported for other cereals, e.g. maize (Ciampitti et al. 2013; Ciampitti & Vyn 2013), with low MnHI than reported here.

The differences in NuHIs among genotypes were due to either AGB differences (e.g. 'Discovery' and 'Reliance'), or the total nutrient accumulated in the straw or the grain. The PHI differences between 'Discovery' and 'Reliance' was a reflection of their differences in AGB and grain yield (Table 3.5), as 'Discovery' had higher AGB and grain yield compared with 'Reliance'. Difference in P accumulation were because 'Discovery' had higher straw P uptake than 'Reliance' (Figure 4.4), thus higher total P uptake. The low PHI for 'Discovery' could be related to its high maximum GLAI and longer LAD (i.e. retention of green area) (Section 3.7.4). This meant 'Discovery' kept more P in its leaves (Section 2.3.3), as one of the key functions of P is the storage and transfer of energy from photosynthesis (McLaren & Cameron 1996; Mengel et al. 2001). However, the low yielding genotype 'Reliance', with a low maximum GLAI and shorter LAD, lost photosynthetic capacity earlier, during grain filling

and translocated more P to the grain. Furthermore, 'Reliance' had a higher P remobilisation efficiency (Section 4.4.5), at 85.0±5.0% compared with 63.0% for 'Discovery', resulting in the same grain P content (Figure 4.4). This may be associated with 'Reliance's earlier leaf senescence and therefore longer remobilisation period. The higher FeHI and MnHI for PFR-3026 compared with PFR-2021 were due to the higher total uptake for PFR-3026 for both nutrients, and lower grain Fe and Mn yield for PFR-2021. This was because PFR-3026 had higher post-anthesis Fe and Mn uptake (Section 4.4.5), which resulted in higher herbage Fe and Mn concentrations (Figure 4.2). This resulted in higher total accumulated Fe and Mn compared with PFR-2021 (Figure 4.5), as they had the same AGB. The higher KHI for PFR-2021 compared with PFR-3019, was because they had the same total K accumulation but PFR-2021 had a higher grain K content (31.7 kg/ha) compared with 24.0 kg K/ha for PFR-3019. The difference in grain K accumulation suggests greater translocation efficiency for PFR-2021. Higher micro-NuHIs (Fe, Mn) for PFR-3026 compared with PFR-2021 meant higher respective nutrients in the grain which is ideal for human nutrition, as micro-nutrient malnutrition (i.e. 'hidden hunger') is increasing globally (Loladze 2002; Myers et al. 2014).

Lower NuHIs for macro-nutrients with increasing N fertiliser supply were because increases in straw nutrient yields were much higher with increasing N fertiliser supply than the increases in grain nutrient yield, e.g. straw K yield increased 42.0% with increasing N fertiliser supply compared with 23.0% for the grain K.

4.6.2 Temporal pattern of nutrient accumulation

In line with the second hypothesis, results showed that nutrient accumulation did not differ in the pre- and post-anthesis period, except for Ca, Cu, K and Mn. There was a near isometric uptake of most nutrient [Fe, Mg, P, S, Zn], with 48.0—57.0% of these nutrients taken up at anthesis. Contrary to our hypothesis, ~87.0% of Cu was accumulated after anthesis, while 74.5—100% of Ca, K, Mn and N, was accumulated before anthesis. Differences in temporal patterns of nutrient accumulation were reported previously for spring (Hocking 1994) and winter wheat (Gaj & Rebarz 2014; Hamnér et al. 2017), and maize (Karlen et al. 1988; Ciampitti et al. 2013; Ciampitti & Vyn 2013). In the current study, the large proportion of Ca and K accumulated during early growth (Table 4.3; Figure 4.4), could be explained by the basal Ca and K fertiliser applied, as the soil Ca and K were low and

borderline, respectively (Table 3.2; Section 3.2.1.1). Gaj & Rebarz (2014) and Hamnér et al. (2017) reported fast accumulation of Ca and K, and attributed that to high availability of Ca and K in the soils. Hocking (1994) reported fast accumulation of K, while accumulation of Ca deviated and amounted to only 69.0% of total uptake at anthesis, possibly due to a different soil type (*Typic Rhodoxeralf*, of sandy clay loam, compared with *Typic Eutrachep,* silt loam, in the current study). Faster accumulation rate for Ca and K during early growth has been reported in other crops, e.g. maize (Bender et al. 2013; Ciampitti et al. 2013; Ciampitti & Vyn 2013) and barley (*Hordeum vulgare* L.) (Rogers et al. 2019). Early uptake is important for these elements, as Ca is a constituent of cell walls in leaves, while K is necessary for the development of lignin and cellulose (McLaren & Cameron 1996) which gives strength and vigour to the plants. In contrast, Malhi et al. (2006) reported no differences in uptake dynamics among K, N and P. In the current study, there was a net loss of K for the low N crops between anthesis and harvest maturity. This has been reported in previous studies and attributed to guttation (Reuter & Robinson 1997; Gaj & Rebarz 2014) followed by wash off of K from foliage by rain water or irrigation (Tukey 1970; LÁSztity et al. 1984; Rogers et al. 2019) and losses from leaf senescence (Rogers et al. 2019).

The near isometric accumulation of most nutrients [Fe, Mg, P, S and Zn] at anthesis was related to the biomass accumulation of 55.0±0.20%. This was lower than the 65.2% (Hocking 1994) reported for spring wheat and 74.4% (Hamnér et al. 2017) for winter wheat. This could help explain some differences between the current study and these previous studies. For example, the low Cu (13.0%) in the current study against the high accumulation (∼76.0%) reported by Hocking (1994). These results show that nutrient uptake after anthesis was substantial for most nutrients, except Ca, K, Mn and N supporting our hypothesis. This novel finding could have implications for nutrient management, as some of these nutrients are highly soluble (e.g. S and N) and easily leached in soils in arable lands (McLaren & Cameron 1996)) and therefore may need to be split applied during the growing season. The application of nutrients based on crop demand will help in controlling nutrient loses to, and polluting the environment.

4.6.3 Optimum N fertilisation rates in relation to the concentration of other nutrients

Contrary to the third hypothesis, results showed that optimum N fertiliser supply resulted in increased concentrations and accumulation for most of the other nutrients at anthesis and harvest maturity. This may be explained by the fact that increased N fertilisation leads to increased allocation of N to the photosynthetic structures (Hawkesford 2014; Hamnér et al. 2017), increasing the photosynthetic capacity of the plant. For the wheat crop to make use of the enhanced photosynthetic capacity (*Loc. cit*), the demand for other nutrients in the tissue also increases, which is in line with the pattern observed for most nutrients in our study. The non-response of the micro-nutrient concentrations to N fertiliser supply at harvest maturity, suggest nutrient deficiencies (*Loc. cit*), however base micro-nutrient levels were not determined in the current study. The increases in macro-nutrient concentration with increasing N fertiliser supply were due to the fact that biomass yield increased with N fertiliser supply (Table 3.5). Where nutrient uptake increased with increasing N fertiliser supply, the proportion of change was greater than the biomass yield increases, e.g. Ca uptake increased by ∼51.0%, compared with ∼31.0% for biomass yield. For all micro-nutrients, the rate of nutrient uptake was proportional to AGB accumulation for both N fertiliser supply rates.

At harvest maturity, the nutrient accumulation differed among the genotypes: 'Discovery' had higher Ca and P, while 'Discovery' and PFR-3019 had higher Ca and PFR-3026 had higher in Fe, Mn and Cu. In all cases, the high accumulation was due to high, respective nutrient concentrations, as all genotypes had the same AGB except for 'Discovery' and 'Reliance'. Furthermore, the grain nutrient accumulation differed among the genotypes (Section 4.4.3.1) for some nutrients. PFR-3019 accumulated higher Ca, P and Mn amounts compared with other genotypes, and in all cases, it had the highest nutrient concentration. In contrast, 'Duchess' had the lowest Ca, P and Mn grain concentrations and hence lower accumulated amounts compared with PFR-3019. PFR-2021 had higher Fe compared with the 'Duchess', PFR-3019 and 'Reliance'. In all cases, PFR-2021 had higher grain concentrations for the respective nutrients, which accounted to the higher nutrient accumulation, as they had the same AGB. This is important for human nutrition, and further studies are needed to confirm whether this is repeatable.

Contrary to the fourth hypothesis, grain K concentration decreased with increasing N_g% (Hamnér et al. 2017). This was attributed to the lower rate of grain K increase with increasing N fertiliser supply (29.0%) compared with the 54.0% for N_g. In contrast to the dilution effect (Jarrell & Beverly 1981), and to the fourth hypothesis, S and Zn grain concentration increased with increasing N_g% (Figure 4.3). Previous studies have shown positive effect of N_g% on Zn (Cakmak et al. 2010) and S (Hamnér et al. 2017) concentration. Nitrogen and S are both constituents of proteins (McLaren & Cameron 1996), while grain proteins have been suggested as a sink for Zn (Cakmak et al. 2010; Kutman et al. 2010). Optimum N can increase the grain S and Zn concentrations by enhancing the grain protein concentration and thereby the sink strength of the grain for S and Zn, and hence the close, positive relationship. The reported interactions between S and N on grain yield and quality parameters (Moss et al. 1981; Randall et al. 1981; Klikocka et al. 2017) indicate that the effects of S nutrition cannot be considered without regard to N. The lower positive ($R^2 \leq 0.21$) relationship for the other nutrients could be attributed to the poor relationship between grain yield and grain size (Figure 3.3 D). This meant that the fourth hypothesis could not be tested for these nutrients, a similar conclusion drawn by Hamnér et al. (2017). The overall positive responses on grain concentrations for all nutrient, except K, indicates that optimised N fertilisation to high yielding wheat genotypes could increase grain concentration of essential nutrients (*Loc. cit*), provided sufficient soil and basal supply (Section 3.2.1.1).

The N: nutrient ratios are a direct function of N uptake and an inverse function of relevant nutrient uptake (Sadras 2006); within a defined range for each N—nutrient (e.g. P) combination. The ratios lower than the range signifies N deficiencies and luxury nutrient uptake, and vive-versa. The N: nutrient ratios increased with developmental stage for the nutrients that were taken up earlier [K, Ca] in the season and were higher at harvest maturity. This was because the crops continued to take up N after K and Ca accumulation had ceased. The decrease of ratios for the other nutrients [e.g. N: P and N: Mg], was an indication that plants were accumulating these nutrients faster than N. The implication was that time of nutrient application should be based on the dynamics of individual nutrient uptake, for example all Ca and K should be applied at sowing. The differences among the genotypes on N: nutrient ratios were either due to higher N uptake. For example, the higher

N: Fe for 'Discovery' and PFR-2021 at harvest maturity was because they took up more N and lower Fe, while PFR-3026 had the lowest N: Fe, N: Mn and N: Cu because of its low N uptake (Section 3.8.1) and the high Fe, Mn and Cu uptake (Section 4.4.3). Overall N: nutrient ratios were higher in the grain (Appendix 4.2) compared with those of the AGB at harvest maturity (Figure 4.6, 4.7), which is consistent with previous reports for N: P and N: K ratios for wheat (Aulakh & Malhi 2005). Furthermore the N: nutrient ranges reported here are consistent with those reported previously, e.g. N: P and N: K (Aulakh & Malhi 2005; Takahashi & Anwar 2007; Gaj & Grski 2014), N: Mg and N: Ca (Gaj & Grski 2014). However, the N: S ratios were lower than the 14—17 reported for wheat (Rasmussen et al. 1975; McGrath et al. 1996; Zhao et al. 1996; Reussi et al. 2011). Lower N: S values means the crops took up more S than N through the growing season, attributed to the either the applied S or S mineralisation, as the initial soil S level was lower than the optimum (Table 3.2).

The overall grain nutrient concentration (Figure 4.1 E, F; 4.2 E, F) were within the reported 'adequate' levels for human health (Reuter & Robinson 1997), except for K (0.36%) and Zn (29.1±1.0 mg/kg) which were lower than the 0.50% (*Loc. cit*) and ≥50 mg/kg (Reuter & Robinson 1997; Liu et al. 2014), respectively. The higher grain K concentration for PFR-2021 compared with the other genotypes needs to be pursued further, as it had the same grain yield as ''Discovery'' (Table 3.5), and higher grain yield compared with the other four genotypes. PFR-2021 accumulated more grain K than the other genotypes, hence the higher concentration. Grain Fe concentration was unaffected by the N treatments, while Zn concentration increased with increasing N fertiliser supply. Cakmak et al. (2010) reported an increase of Fe and Zn concentration with increasing grain N concentration. The Fe concentration could be increased by Fe soil or foliar fertiliser application as reported before by these same authors.

4.7 Conclusions

The NuHIs differed widely among nutrients, genotypes (except for ZnHI and CuHI) and with increasing N fertiliser supply (except for PHI and all micro-nutrients). The NuHIs ranged from low (≤0.30) for CaHI, CuHI, FeHI and KHI, moderate (0.30—0.60) for MnHI, SHI and ZnHI and high (>0.60) for MgHI, NHI and PHI, reflecting individual nutrient phloem mobility. In the herbage, a higher concentration of N resulted in increased concentrations for the macro-nutrients, but no response for the micro-nutrients. Implication was that crops were

accumulating micro-nutrients at the same rate as AGB accumulation for both N fertiliser rates. Nutrient accumulation was split evenly pre- and post-anthesis, except for Ca, K and Mn that were accumulated earlier in the growing season, and Cu that was accumulated later during growth. In the grain, N fertiliser supply had no major impact on nutrient concentration except for S and Zn (close, positive relationship to N_g%) and K (negative relationship to N_g%). The N: nutrient ratios increased with developmental stage for the nutrients that were taken up earlier [K, Ca] in the season and were higher at harvest maturity, but decreased for the other nutrients [e.g. N: P and N: Mg], was an indication that plants were accumulating these nutrients faster than N. The implication was that time of nutrient application should be based on the dynamics of individual nutrient uptake, for example all K and Ca applied at sowing.

Chapters 3—4 have described the effects of genotype, N fertiliser supply and their interactions on CHI, NHI, NuHIs and NUE. The effects of genotype, N fertiliser supply and their interactions will be further explored in a glasshouse experiment (Experiment 2) and this will be used to confirm the results and genotype rankings established in Experiment 1 (Chapters 3—4). As there are no established N to micro-nutrients relationship in literature, the values reported here will be confirmed in the next two controlled experiments Chapters 5 & 6).

Chapter 5: Crop and nutrient harvest indices for six spring sown wheat genotypes grown at low or optimum nitrogen fertiliser supply in a Glasshouse.

5.1 Introduction

Field experiment results (Chapters 3 & 4), showed genotypic differences in CHI, NHI, NuHIs and NUE. In wheat, a large part of the biomass and N harvested in the grain originates from remobilisation from vegetative organs during the post-anthesis period (Andersson et al. 2005; Allard et al. 2013). Biomass and N remobilization from aboveground organs have been extensively studied (e.g. Austin 1977, 1980; Peltonen-Sainio et al. 1997; Fageria 2014) and were confirmed in Experiment 1. However, reports on accumulation, partitioning and remobilisation of other nutrients for spring sown wheats are limited and/ or dated (Miller et al. 1993; Hocking 1994) or reported for winter wheats (Hamnér et al. 2017). The absence of comprehensive and recent data on the nutritional needs of modern spring sown wheat production systems necessitates an understanding of their nutrient uptake, partitioning, and remobilisation. The null hypothesis is that CHI, NHI, NuHI and NUE will not differ among the spring sown wheat genotypes, at low or optimum N fertiliser supply.

The aim of Experiment 2 (Chapter 5) is to confirm genotype ranking on (1) CHI, NHI, NuHI and NUE reported in Experiment 1 (Chapters 3 and 4), and (2) the N to nutrients ratios established in Chapter 4. The objective is to determine the effects of genotype, N fertiliser supply and their interaction on CHI, NHI, NuHIs, NUE and N: nutrient ratios for six spring wheat genotypes, and to confirm genotype rankings reported in Experiment 1.

5.2 Materials and Methods

Experiment 2 was carried out in a glasshouse (Aluminex House; 43°38'43"S 172°27'44"E) at the Nursey Greenhouse Centre, Lincoln University, New Zealand in 2018-2019 season.

5.2.1 Experiment design and treatments

Experiment 2 was a randomised complete block design, replicated six times. The treatments consisted of a combination of six wheat genotypes (Section 1.4; Appendix 1.1) and two rates of N fertiliser supply: low (85) and optimum (285) kg N/ha. The N fertiliser supply rates were similar to the total N in Experiment 1 (fertiliser + soil N to 1.2 m depth) (Section 3.2.2).

However, the genotype PFR-2021 reached reproductive development earlier than the other five genotypes, and birds ate many of the developing grains before experiment was covered with nets. It was therefore discarded from analyses.

The genotypes were grown in a 60% sieved composted bark (4 mm diameter) and 40% pumice (3 mm diameter) mix, in 80 cm long PVC tubes, with an inner diameter of 15 cm (total surface area = 176.8 cm^2 or 0.018 m^2). The base of each tube was covered with perforated 1 L pots, pushed in upside down, to allow free drainage. The PVC tubes were positioned on solid 4 L pail containers to capture all the mineral solution draining through the bottom. The tubes were filled with 14.1 kg of the potting mix. The crops were watered on alternate days, which allowed the drained mineral solution captured in the pails to be returned to the respective tubes between watering events. Eight seeds were sown per tube (20 mm depth) on 8 September 2018, and thinned to five seedlings, one week after emergence. This translates to 280 plants per m^2, which was similar to the population established in the field in Experiment 1 (Section 3.2.3.1).

The potting mix was analysed for fertility, and results showed: pH 6.1, nitrate-N 1 mg/L, ammonium-N <1 mg/L, Olsen P 6 mg/L, sulphate-S <1 mg/L, K 39 mg/L, Mg 2 mg/L, Ca 3 mg/L and Na 8 mg/L potting mix. These values are lower than the optimum needed for crop production (Table 3.2), except for pH, K and Na. Therefore, base fertilisers, with some modification for N, were added during potting mix preparation at rates reported previously (Nguyen et al. 2017). The application rates were: 0.021 g/L osmocote (38-0-0), 0.30 g/L superphosphate (0-9-0-11-0-20), 0.30 g/L horticultural lime (primarily calcium carbonate), and 0.30 g/L Osmocote (0-0-37), 0.3 g/L Micromax® (6% Ca, 3% Mg, 12% S, 0.1% B, 1% Cu, 17% Fe, 2.5% Mn, 0.05% Mo and 1% Zn) and 1 g/L Hydraflo (wetting agent). An additional 0.03 g/L (equivalent to 50 kg N/ha) was applied to all tubes 3 weeks after emergence. The additional N brought the total basal N fertility to the same levels of mineral N measured in Experiment 1 (85 kg N/ha; Table 3.2).

The optimum N fertiliser rate was applied over three times during the season, at rates equivalent to 100 kg N/ha (~ 0.18 g N/tube) at the start of tiller formation (GS21), and 50 kg N/ha each, at appearance of the second node (GS32) and flag leaf (GS39), as urea (46% N) to the respective tubes. The urea was dissolved in 8 L of water, and 110 mL of solution was applied per tube, followed by light irrigation.

As the crop matured, the whole experimental area was covered with bird netting, 2.0 m above the floor from 27 November 2018 to maturity, to prevent birds from eating the maturing grain and ensure accuracy of yield results. Plants were sprayed once for aphids using pilarking (a.i. 200 g/L imidacloprid, SC and 750 g/L methylene chloride), at a rate of 3.5 mL per 5 L of water on 10 December 2018.

5.2.2 Measurements

Experiment 2 was harvested twice during the growing season, at 50% anthesis (70 DAE) and harvest maturity (130 DAE). At anthesis, the shoots were partitioned into stem + sheath, leaf lamina and ears. At harvest maturity, the ears were hand thrashed to separate the grain from the chaff, after which the grain sample was further cleaned. Green leaf area per tube was determined from the partitioned leaf lamina at anthesis, using a Licor 3100 area meter (Licor Inc, NE, USA). The partitioned material was then dried in a forced air oven at 60°C until constant weight for ~72 hours, and weighed. These samples were ground with a Cyclone Sample Mill (Udy Corporation, Fort Collins, Colorado, USA) to pass through a 1 mm screen and placed in plastic vial for storage.

The samples were analysed for N amount and mineral nutrients (Sections 3.5, and 4.2.1). Briefly, N was determined by automated dry combustion-gas chromatography with a Vario Max CN Macro Elemental Analyser (Elementary GmbH, Hanau, Germany) (Section 3.5.1). Other nutrients (Figure 2.3) were analysed using the ICP – OES (Agilent Australia Pty Ltd, Vic, Australia) (Section 4.2.1.1) at the Lincoln University Laboratory. Total nutrient yields (mg/tube) were calculated as the product of DM yield (g per tube) and the nutrient concentration (mg/kg DM) in the harvested crop (Muchow 1988; Subedi & Ma 2005).

The grain yield was adjusted to a standard 14% moisture content (86% DW). The total grain weight was recorded, but as the number of grains were <1,000 per tube, the TGW was calculated as a quotient of grain weight and number of grains, multiplied by 1,000.

5.2.3 Statistical analyses

Biomass, grain yield, nutrient accumulation and partitioning responses were analysed as described in Section 3.6, using a mixed model approach, fitted with REML programme in Genstat 18th edition. Briefly, differences associated with treatment means were separated by $LSD_{5\%}$, with associated d.f. Fixed effects in the models were genotype, N fertiliser supply,

date and all interactions and random effects accounted for the position (block+column) within the experiment. A correlation structure was modelled for date to account for repeated measures. Each variable was analysed separately. When there were interactions of treatments, variation ratios greater than 10 meant the main effects dominated the discussion. All the calculated parameters have been described in previous sections: Sections 2.1 (CHI), 2.3 (NHI, NuHI and NUE) and 3.6 (PANU, NR and NRE). Unless otherwise stated, interactions are given in the text when they are significantly different ($P \leq 0.05$).

5.3 Results

5.3.1 Crop harvest index (CHI)

The CHI differed among the genotypes and was lower for Duchess at 0.33, compared with 0.44±0.04 for the other genotypes (Table 5.1). Furthermore, CHI decreased ($P = 0.001$) with N supply, from 0.46±0.03 for the low N crops to 0.38 when 285 kg N/ha was applied. This contrasts CHI results from Experiment 1 (Section 3.7.2). The low CHI for 'Duchess' was attributed to the high AGB and low grain yield (Table 5.1). The low grain yield was associated with a low TGW (Table 5.2).

There were interactions ($P \leq 0.02$) between N fertiliser supply and genotype for total shoot biomass at anthesis and harvest maturity (Table 5.1). This was because shoot biomass did not differ among the genotypes at low N fertiliser supply, and averaged 6.66±0.67 g at anthesis and 13.2±2.10 g at harvest maturity. In contrast, shoot biomass differed ($P < 0.001$) among the genotypes when 285 kg N/ha was applied. 'Discovery' had the highest ($P < 0.001$) total shoot biomass yield at anthesis and harvest maturity, and 'Reliance' had the lowest biomass yield at anthesis and harvests maturity. Total shoot biomass for the other genotypes of 17.2±1.1 g at anthesis and 40.5±2.1 g at harvest maturity was intermediate. These results confirmed Experiment 1 rankings (Section 3.7.2).

At anthesis, shoot biomass increased from 6.66±0.67 g for the 85 kg N/ha crops to 18.6 g/tube when 285 kg N/ha was applied (Table 5.1). At harvest maturity, shoot biomass increased from 13.2±1.3 g to 40.1 g/tube for the 85 kg N/ha and 285 kg N/ha, respectively.

Table 5.1: Total shoot biomass[1,2] (g) at anthesis, the straw (g) and grain biomass (g) at harvest maturity and the crop harvest index (CHI) for five wheat genotypes grown at low (85 kg/ha) and optimum (285 kg/ha) nitrogen (N) fertiliser supply in a Glasshouse facility at Lincoln, New Zealand, during 2018-2019 season.

Treatments		Anthesis biomass	Harvest maturity biomass		
N fertiliser	Genotypes	Shoot (g)	Straw (g)	Grain (g)	CHI (g/g)
85 kg/ha	'Discovery'	7.19_{a}	7.68_{a}	7.70_{a}	0.50_{a}
	'Duchess'	6.29_{a}	11.0_{a}	5.17_{a}	0.35_{b}
	PFR-3019	5.87_{a}	5.22_{a}	5.90_{a}	0.51_{a}
	PFR-3026	6.93_{a}	5.61_{a}	5.77_{a}	0.51_{a}
	'Reliance'	7.00_{a}	6.46_{a}	5.36_{a}	0.46_{ab}
	Mean	*6.66_{B}*	*7.18_{B}*	*5.98_{B}*	*0.46_{A}*
285 kg/ha	'Discovery'	25.1_{a}	32.9_{a}	16.5_{a}	0.33_{b}
	'Duchess'	21.9_{a}	29.5_{a}	13.4_{b}	0.31_{b}
	PFR-3019	13.2_{c}	22.3_{b}	16.8_{a}	0.43_{a}
	PFR-3026	16.4_{b}	22.1_{b}	17.3_{a}	0.44_{a}
	'Reliance'	16.3_{bc}	19.5_{b}	10.9_{c}	0.37_{ab}
	Mean	*18.6_{A}*	*25.1_{A}*	*15.0_{A}*	*0.38_{B}*
Source of variance: P value ($LSD_{5\%}$)					
N fertiliser supply (N)		<0.001 (1.40)	<0.001 (2.32)	<0.001 (1.47)	0.001 (0.05)
Genotype (G)		<0.001 (2.22)	<0.001 (3.66)	0.01 (2.33)	0.01 (0.08)
N*G		<0.001 (3.13)	0.02 (5.18)	0.10 (3.30)	0.50 (0.12)

[1]Means with letter subscripts in common, within a column are not different at $\alpha = 0.05$

[2]Total shoot biomass at harvest maturity is the sum of straw and grain yield.

The shoot biomass at anthesis was a reflection of the leaf area of the different genotypes (Figure 5.1). The green leaf area per tube was affected by the interaction ($P < 0.001$) between N fertiliser supply and genotype (Figure 5.1), as there were no differences among the genotypes at low N fertiliser supply at an average of 240±140 cm^2 per tube. In contrast, leaf area differed ($P = 0.002$) among the genotypes when 285 kg N/ha was applied. 'Discovery' and 'Duchess' had higher leaf area at 2216±140 cm^2 per tube compared with 1260 cm^2 for the other genotypes.

Grain yield differed ($P < 0.001$) among the genotypes (Table 5.1). 'Reliance' had the lowest average grain yield at an average of 8.11±0.80 g/tube and 'Discovery' had the highest average grain yield at 12.1 g/tube. The other genotypes were intermediate at an average of 10.7 g/tube. These differences in grain yield also confirmed the rankings in Experiment 1 (Section 3.7.2). The low grain yield for 'Reliance' was attributed to the low number of grains

and moderate TGW (Table 5.2). Furthermore, the low grain yield for 'Duchess' and PFR-3019 was due to a low TGW. Grain yield increased ($P < 0.001$) with increasing N fertiliser supply from an average of 5.98±0.70 g/tube for the 85 kg N/ha crops to 15.0 g/tube when 285 kg N/ha was applied.

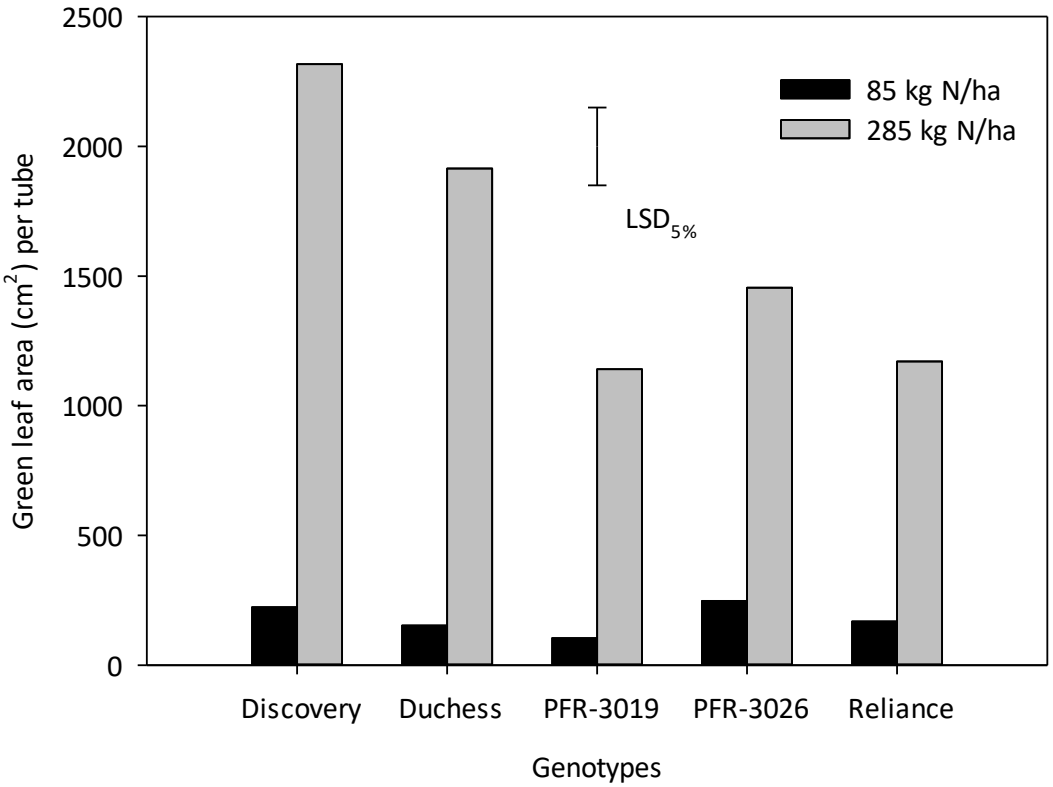

Figure 5.1: Leaf area per tube for the different genotypes grown at low (85 kg N/ha) or optimum (285 kg N/ha) fertiliser supply at anthesis, in a Glasshouse at Lincoln, New Zealand in 2018-19

The AGB at anthesis was on average 50±0.03% of the total biomass at harvest maturity (Table 5.1) and was unaffected ($P \geq 0.11$) by the treatments.

5.3.2 Yield components

5.3.2.1 Numbers of grains per unit area (grain density)

There was an interaction ($P = 0.002$) between N fertiliser supply and genotype on the number of grains per tube (grain density) (Table 5.2; Figure 5.2 B, D). There were no differences in grain density among the genotypes for the 85 kg N/ha crops, at an average of

126±11.7 grains/tube but when 285 kg N/ha was applied, PFR-3019 produced the highest number of grains at 466 grains/tube and 'Reliance' at 228 grains/tube had the lowest. The number of grains trebled to 362±16.0 grains/tube when 285 kg N/ha was applied compared with the low N crops.

Grain density in the glasshouse (Table 5.2) was 11-42% lower than Experiment 1. For example, the average of 232 grains per tube for 'Discovery' gives 13,107 grain per m^2 compared with the average of 16,104 grains per m^2 in Experiment 1.

Table 5.2: Grain density[1,2] and thousand grain weight (TGW; g) for five wheat genotypes grown at low (85 kg/ha) and high (285 kg/ha) nitrogen (N) rate in a Glasshouse facility at Lincoln, New Zealand, during 2018-2019 season.

Genotype	Grain density		Thousand grain weight (g)	
N fertiliser	85 kg/ha	285 kg/ha	85 kg/ha	285 kg/ha
'Discovery'	126 (7,000)$_a$	338 (18,778)$_{bc}$	52.0$_a$	49.2$_a$
'Duchess'	130 (7,222)$_a$	390 (21,667)$_{ab}$	42.3$_{ab}$	34.9$_b$
PFR-3019	123 (6,833)$_a$	466 (25,889)$_a$	37.7$_b$	36.3 $_{ab}$
PFR-3026	121 (6,722)$_a$	386 (21,444)$_b$	48.5$_{ab}$	46.1$_a$
'Reliance'	131 (7,222)$_a$	228 (12,668)$_c$	37.5$_b$	42.9$_{ab}$
Mean	126 (7,000)$_B$	362 (20,111)$_A$	43.6$_A$	41.9$_A$
Significance: P value ($LSD_{5\%}$)				
N fertiliser	<0.001 (34.6)		0.53 (5.70)	
Genotype (G)	0.005 (54.7)		0.02 (9.01)	
N*G	0.003 (77.4)		0.68 (12.8)	

[1]Means with letter subscripts in common, within a column are not different at α = 0.05

[2]Numbers in parenthesis are the equivalent per m^2, using a conversion from a surface area of 0.018 m^2 (Section 5.2.1).

5.3.2.2 Thousand grain weight (TGW)

The TGW was unaffected (P = 0.53) by N supply at an average of 42.7±2.7 g (Table 5.2). However, TGW differed (P = 0.02) among the genotypes, being higher for 'Discovery' and PFR-3026 at 49.0±4.30 g compared with an average of 38.6 g for the other genotypes. The TGW for the glasshouse were consistent with Experiment 1, for example, TGW for 'Discovery' of 50.6±4.30 g in glasshouse (Table 5.2) was same as the 50.0±1.4 g in Experiment 1 (Figure 3.4).

The relationship between TGW and grain density was poor (R^2 = 0.08) and negative (Figure 5.2 D), because TGW was unaffected by N supply, and the grain density at low N fertiliser

supply did not differ among the genotypes (Table 5.2). Grain density explained 90% of the variation in grain yield (Figure 5.2 B), compared with 0.10% for TGW (Figure 5.2 C). However, when the relationship between grain yield and TGW was separated on N fertiliser supply, there was a moderate relationship (R^2 = 0.52) for the 85 kg N/ha crops, but none (R^2 = 0.06) for the 285 kg N/ha crops. This relationship was in contrast to Experiment 1.

5.3.3 Nitrogen harvest index (NHI)

The NHI differed (P = 0.03) among the genotypes (Figure 5.4 C, D), being lower for 'Discovery' and 'Duchess' (0.72±0.04) compared with 0.78 for the other genotypes. This was because 'Discovery' and 'Duchess' had higher total shoot N yield (Section 5.3.3.2) compared with the other genotypes, but had similar N_g yield to the other genotypes (Section 5.3.3.3) except for 'Reliance'. These results also confirmed genotype ranking in Experiment 1 (Section 3.8.3).

The NHI decreased by ~6.40% with N supply, from 0.78±0.02 for the 85 kg N/ha crops to 0.73 when 285 kg N/ha was applied, because shoot N yield increased 8-fold with N fertiliser supply, compared with 3-fold for the N_g yield.

5.3.3.1 Shoot nitrogen concentration (N%)

Shoot N% decreased ($P < 0.001$) with developmental stage (Figure 5.3), from 1.71±0.03% at anthesis to 1.10% at harvest maturity. At anthesis, shoot N% differed ($P < 0.001$) among the genotypes, being higher for PFR-3019 and PFR-3026 at 1.90±0.05% compared with 1.58% for the other genotypes. At harvest maturity, shoot N% also differed (P = 0.02) among the genotypes, higher for PFR-3019, PFR-3026 and 'Reliance' at 1.12±0.07% compared with 0.95% for the other genotypes.

At anthesis, shoot N% increased with increasing N fertiliser supply from 0.79±0.03% for the 85 kg N/ha crops to 2.63% when 285 kg N/ha was applied. However, at harvest maturity shoot N% was unaffected (P = 0.25) by N fertiliser supply, at an average of 1.10±0.03%.

5.3.3.2 Grain N concentration (N_g%)

There was an interaction (P = 0.03) between N fertiliser supply and genotype for the N_g% (Figure 5.3 E, F), because N_g% for PFR-3019 and 'Reliance' did not differ with N fertiliser supply at 2.12±0.03%. In contrast, N_g% increased with N supply for the other genotypes,

from 1.74±0.03% for the 85 kg N/ha crops to 2.11% when 285 kg N/ha was applied. The N_g% differed among the genotypes, being lower for 'Discovery' and PFR-3026 at an average of 1.85±0.05, compared with 2.11% for the other genotypes, corresponding to a GPC of 10.6—12.0%. A higher N_g% for Reliance and PFR-3019 and lower N_g% for 'Discovery' and PFR-3026 were also reported in Experiment 1 (Section 3.8.2).

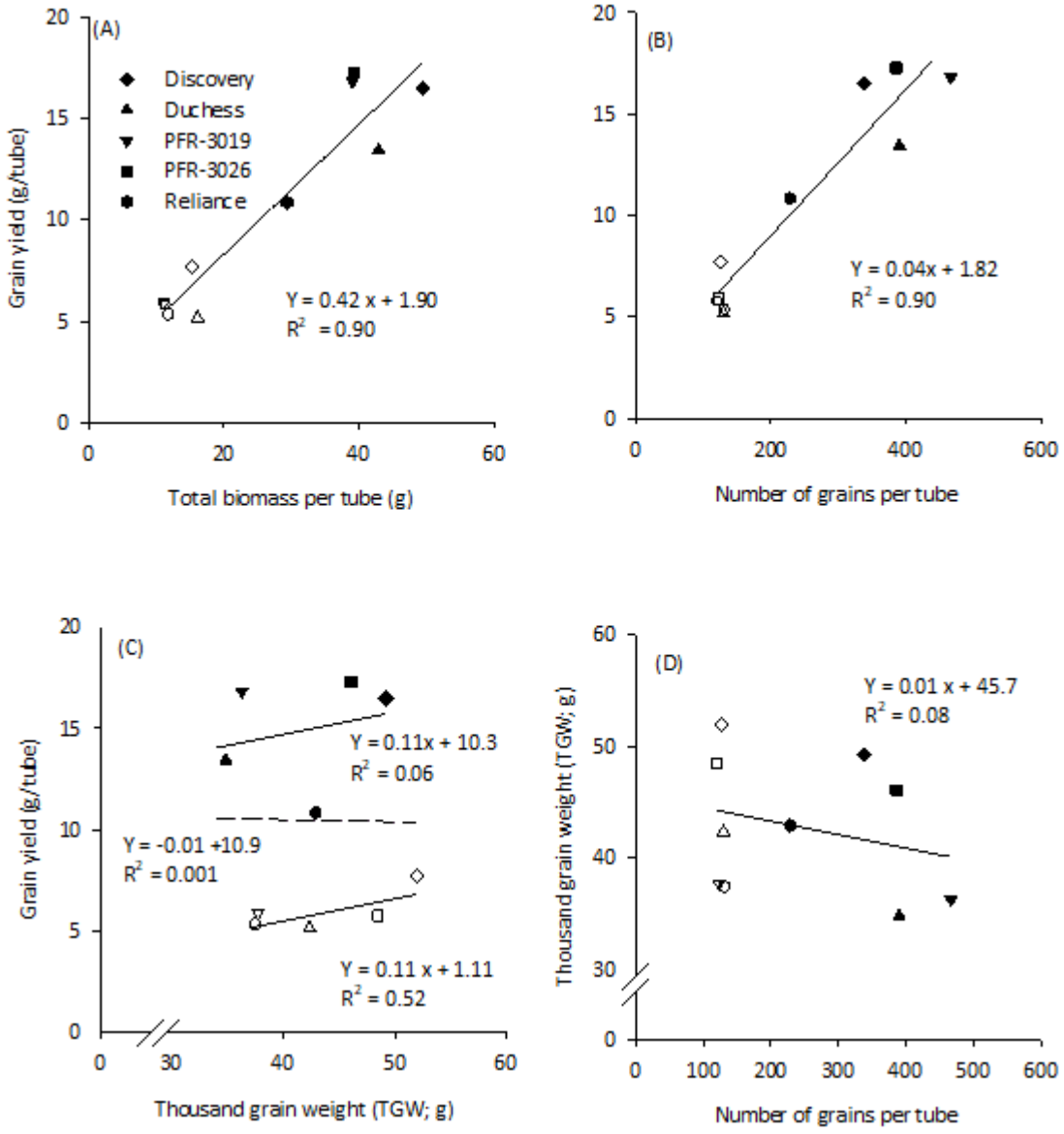

Figure 5.2: Grain yield per tube against: (A) total biomass, (B) grain density and (C) thousand grain weight (TGW, g) [the dotted line represent the combined data], and (D) TGW against grain density for five wheat genotypes grown at low (85 kg N/ha; open symbols) and optimum (285 kg N/ha; closed symbols) nitrogen (N) in a Glasshouse at Lincoln, Canterbury, New Zealand in 2018-2019 season.

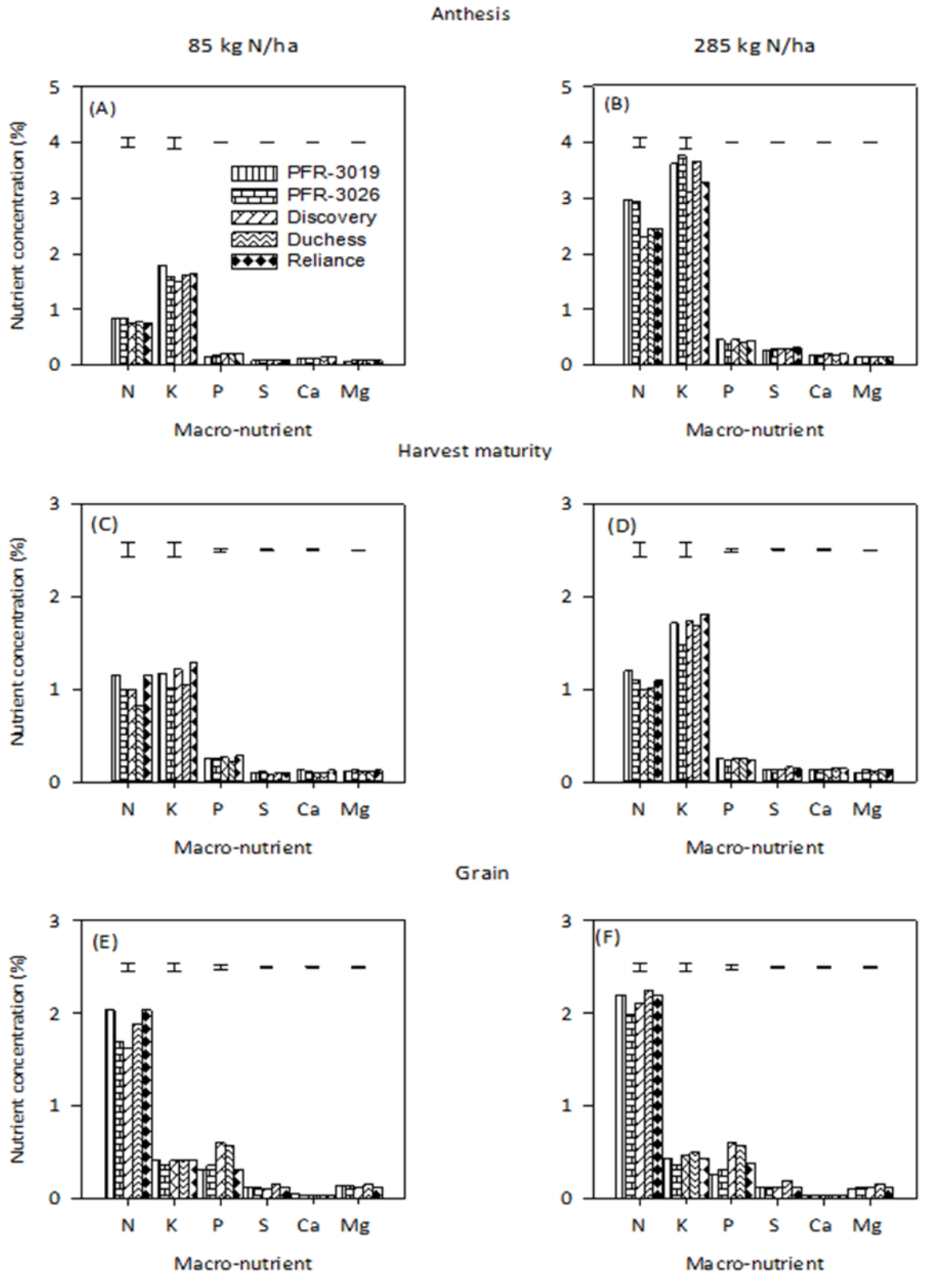

Figure 5.3: Weighted shoot (A—D) and grain (E—F) macro-nutrient concentration (%) at anthesis (A and B) and harvest maturity (C and D) and for five wheat genotypes grown at low (85 kg; A, C, E) or optimum (285 kg; B, D, F) N fertiliser supply (kg/ha) at Lincoln, Canterbury in 2017-18 season. Vertical bars are the leaf significant differences ($LSD_{5\%}$) for the genotypes at each nitrogen levels

5.3.3.3 Nitrogen accumulation (mg/tube)

There were interactions ($P < 0.05$) between N fertiliser supply and genotype on total shoot N yield at anthesis and straw N yield at harvest maturity (Figure 5.4). This was because total N yield did not differ among the genotypes at 85 kg N/ha but did ($P < 0.001$) at 285 kg N/ha at anthesis (Figure 5.4 B) and harvest maturity (Figure 5.4 D). 'Discovery' and 'Duchess' had higher ($P \leq 0.03$) N yield compared with the other genotypes at anthesis and harvest maturity. For example, at anthesis, total shoot N yield was 621±33.0 mg/tube for 'Discovery' and 'Duchess' compared with 478 mg/tube for the other genotypes. At harvest maturity, total shoot N yield was highest for 'Discovery' and PFR-3019 at 488±25.0 mg/tube, and lowest for 'Reliance' at 330 mg. The other genotypes were intermediate at 442 mg/tube. Similarly, the straw N yield (Figure 5.4 D) for 'Discovery' and 'Duchess' was 152±12.0 mg/tube compared with 97 mg for the other genotypes at harvest maturity. These differences were attributed to the higher leaf area for 'Discovery' and 'Duchess' (Figure 5.1) compared with the other genotypes.

At anthesis, total N accumulation increased ($P < 0.001$) with increasing N fertiliser supply, from 65.0±21.0 mg/tube for the 85 kg N/ha crops to 536 mg when 285 kg N/ha was applied (Figure 5.4 A, B). At harvest maturity (Figure 5.4 C, D), shoot N yield increased from 134±16.0 for the 85 kg N/ha crops to 438 mg/tube when 285 kg N/ha was applied.

5.3.3.4 Grain N accumulation (mg/tube)

There was an interaction ($P < 0.001$) between N fertiliser supply and genotype on N_g yield (Figure 5.4 C, D), as the N_g yield did not differ among the genotypes for the 85 kg N/ha crops, at an average of 104±32.0 mg. In contrast, N_g yield differed among the genotypes when 285 kg N/ha was applied. It was 239±14.0 mg for 'Reliance' compared with an average of 339 mg for the other genotypes.

5.3.3.5 Specific leaf nitrogen (SLN; g N/m^2)

At anthesis, the SLN differed ($P = 0.005$) among the genotypes (Appendix 3.3), highest for 'Duchess', 'Reliance' and PFR-3019 at 2.0±0.19 g N/m^2 compared with 1.54 g N/m^2 for Discovery and PFR-3026. The SLN increased ($P < 0.001$) with N fertiliser supply, from 1.53 g N/m^2 for the 0 kg N/ha crops to 2.10 g N/m^2 for the optimum N fertiliser crops. As the total leaf N did not differed among the genotypes, the SLN values reported here were

determined by the green leaf area (Figure 5.1), higher for the genotypes with low green leaf area and vice-versa (Appendix 3.3).

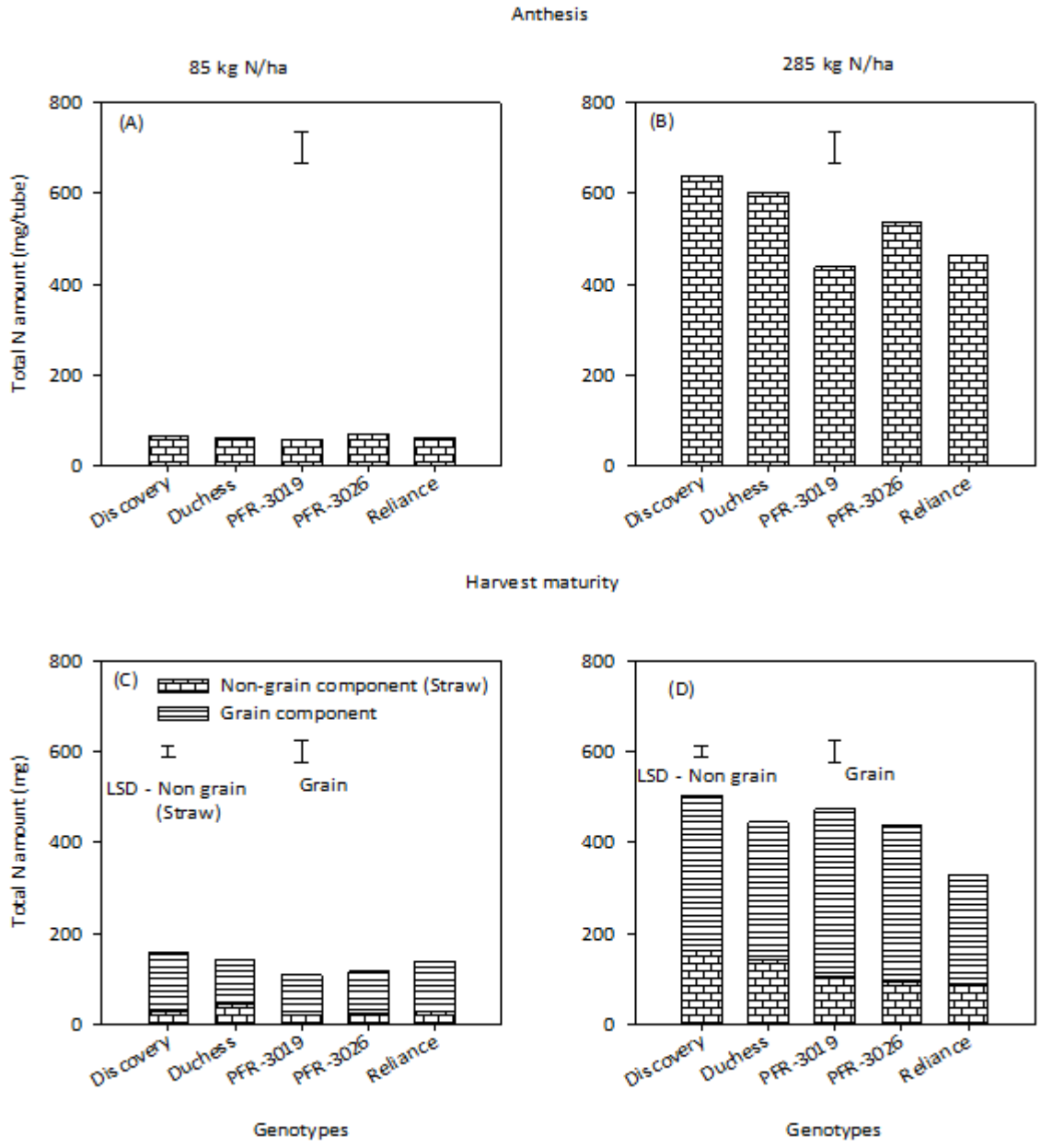

Figure 5.4: Total nitrogen accumulation (mg) for five wheat genotypes grown at low (85 kg/ha; A, C) and optimum (285 kg/ha, B, D) nitrogen (N) rate in a Glasshouse at Lincoln, Canterbury, New Zealand in 2018-2019 season at anthesis (A & B) and harvest maturity (C & D) growth stages. Vertical line are the least significant differences (LSD5%) for the genotypes.

5.3.4 Nitrogen remobilisation and use efficiencies

5.3.4.1 Nitrogen remobilisation (NR) and post-anthesis N uptake (PANU)

There were interactions ($P \leq 0.03$) between N fertiliser supply and genotypes for NUE and NutE (Table 5.3). This was because NUE did not differ between N fertiliser supply for PFR-3019 (48.3±2.40) and PFR-3026 (50.2). In contrast, NUE decreased with increasing N supply for the other genotypes. For example, NUE for 'Discovery' decreased from 63.6±1.51 at low N fertiliser supply to 45.4 when 285 kg N/ha was applied. The NutE for 'Reliance' was 36.2±1.91 for both N fertiliser supply rates. In contrast, NutE decreased with increasing N supply for the other genotypes. For example, NutE for 'Discovery' decreased from 49.4 for the low N fertiliser supply to 32.5 when 285 kg N/ha was applied.

Table 5.3: Mean[1] nitrogen uptake, utilisation and use efficiency (NupE, NutE, NUE), remobilisation (NR) and post-anthesis N uptake (PANU) for five wheat genotypes grown with low (85 kg) or optimum (285 kg) N/ha in a Glasshouse at Lincoln, New Zealand, in 2017-2018 season.

Treatments		N efficiencies (kg/kg), remobilisation (mg) and PANU (kg/ha)				
N fertiliser	Genotype	NUE	NupE	NutE	NR	PANU
85 kg N/ha	'Discovery'	63.6_{a}	1.46_{a}	49.4_{a}	36.9_{a}	90.3_{a}
	'Duchess'	47.8_{b}	1.32_{a}	37.2_{b}	38.5_{a}	65.8_{ab}
	PFR-3019	50.3_{b}	1.01_{a}	40.4_{b}	32.5_{a}	53.3_{ab}
	PFR-3026	52.7_{b}	1.08_{a}	49.2_{a}	53.2_{a}	40.5_{b}
	'Reliance'	49.6_{b}	1.28_{a}	39.0_{b}	35.1_{a}	73.6_{ab}
	Mean	*52.8_{A}*	*1.24_{A}*	*43.0_{B}*	*39.2_{B}*	*64.7_{A}*
285 kg N/ha	'Discovery'	45.4_{a}	1.39_{a}	32.5_{b}	478_{a}	-136_{a}
	'Duchess'	37.5_{b}	1.22_{a}	30.1_{b}	460_{a}	-158_{a}
	PFR-3019	46.3_{a}	1.30_{a}	35.5_{ab}	335_{a}	34.7_{a}
	PFR-3026	47.6_{a}	1.21_{a}	39.4_{a}	439_{a}	-96.0_{a}
	'Reliance'	32.1_{c}	1.03_{a}	33.4_{b}	374_{a}	-135_{a}
	Mean	*41.8_{B}*	*1.23_{A}*	*34.2_{A}*	*417_{A}*	*-98.1_{B}*
	Significance: P value ($LSD_{5\%}$)					
	N fertiliser	0.002 (3.18)	0.80 (0.14)	<0.001 (2.54)	<0.001 (43.3)	<0.001 (45.2)
	Genotype (G)	0.006 (5.02)	0.10 (0.22)	<0.001 (4.01)	0.18 (68.5)	0.10 (71.4)
	G*N	0.020 (7.10)	0.22 (0.31)	0.03 (5.67)	0.15 (96.8)	0.04 (101)

[1]Means with letter subscripts in common, within a column are not different at $\alpha = 0.05$

The NUE and NutE also differed among the genotypes, and 'Discovery' had higher NUE at ~ 54.5 compared with ~ 41.6±2.40 for 'Duchess' and 'Reliance'. The other genotypes were intermediate at ~ 49.3. The NutE was higher for 'Discovery' and PFR-3026 at an average of

42.6±1.90 compared with 35.9 for the other genotypes. These results highlight the same ranking as Experiment 1 (Section 3.8.4, Table 3.9). However, NR and PANU did not differ (P = 0.18; 0.10, respectively) among the genotypes.

The NUE, NutE, N remobilisation and PANU were affected (P ≤ 0.002) by N supply (Table 5.3). For example, NUE decreased with increasing N fertiliser supply, from 52.8±2.90 at low N fertiliser supply to 41.8 when 285 kg N/ha was applied. In contrast, NR increased with increasing N supply from 39.2±32.0 for the low N fertiliser supply crops to 417 when 285 kg N/ha was applied.

However, NupE was unaffected (P = 0.84) by N fertiliser supply and did not differ (P = 0.10) among the genotypes (Table 5.3). This meant the differences in NUE were attributed to the NutE. The relationship between NUE and NutE was closer (R^2 = 0.65) compared with NupE (R^2 = 0.15).

There was a positive relationship (R^2 = 0.50—0.60) between NutE and NUE at both N fertiliser rates (Table 5.4). However, the relationship between NupE and NUE was inconsistent, stronger (R^2 = 0.61) at the optimum N fertiliser supply but non—existent at low N fertiliser supply. There was also no relationship (R^2 = 0.02—0.07) between NupE and NutE at both N fertiliser rates.

Table 5.4: The relationship between nitrogen use efficiency (NUE) and nitrogen uptake and utilisation efficiencies (NupE, NutE), and NupE and NutE for wheat crop genotypes grown with 85 or 285 kg N/ha in a Glasshouse at Lincoln, New Zealand, in 2017-2018 season.

N Efficiency	N fertiliser	NupE	NutE
NUE	85 kg/ha	Y = 0.01x + 0.78; R^2 = 0.05	Y = 0.80x +1.04; R^2 = 0.59
	285	Y = 0.01x + 0.651; R^2 = 0.61	Y = 0.30x + 21.8; R^2 = 0.50
NupE	85		Y = -6.93x + 51.6; R^2 = 0.07
	285		Y = 4.61x +28.5; R^2 = 0.02

5.3.5 Accumulation and partitioning of other macro- and micro-nutrients

5.3.5.1 Nutrient harvests indices (NuHI)

There were interactions (P ≤ 0.01) between N fertiliser supply and genotype for MnHI and CuHI (Figure 5.8; Appendix 5.2), as they were unaffected by N fertiliser supply for some but not all genotypes. For example, CuHI for ‘Discovery’ and ‘Duchess’ decreased with increased

N supply, but was unaffected by N fertiliser supply for the other genotypes. Furthermore, MnHI decreased with increasing N fertiliser supply for PFR-3019 and increased with increasing N fertiliser supply for PFR-3026, but was unaffected by N supply for the other genotypes.

All NuHIs differed ($P \leq 0.003$) among the genotypes except for MgHI, FeHI and ZnHI. This was because, grain Mg and Zn yield did not differ ($P \geq 0.25$) among the genotypes (Section 5.3.4.4), while total shoot Fe yield did not differ among genotypes (Section 5.3.4.1). Overall, PFR-3019 had higher PHI and SHI, while 'Discovery' had higher KHI and PFR-3026 had higher CaHI. The differences in NuHIs among genotypes were mostly due to the fact that the genotype (PFR-3019, 'Discovery' and PFR-3026) with higher NuHIs had consistently higher grain accumulation for the respective nutrients, e.g. PFR-3019 had higher P and S accumulation compared with the other genotypes, coupled with lower total nutrient accumulation (Figure 5.7). Similarly, higher KHI for 'Discovery' was due to high grain K yield and moderate total K uptake. All NuHIs decreased ($P \leq 0.003$) with increasing N fertiliser supply, except for PHI, MnHI and ZnHI, which were unaffected ($P \geq 0.18$) by N fertiliser supply. The KHI decreased from 0.15±0.01 for the 85 kg N/ha treatments to 0.09 when 285 kg N/ha was applied, while CuHI decreased from 0.30±0.03 for the 85 kg N/ha crops to 0.15 when 285 kg N/ha was applied.

5.3.5.2 Shoot nutrient concentration (%; mg/kg)

Average herbage concentration responses to developmental stage differed ($P < 0.001$) with nutrient (Figure 5.3; 5.5): decreased ($P < 0.001$) towards maturity for N, P, K, Ca and S and increased ($P \leq 0.01$) towards maturity for Mg, Mn, Zn and Cu, but did not change ($P = 0.33$) for Fe. This is a reflection of the proportion of nutrient uptake at anthesis (Section5.3.4.5). The decrease in concentration towards maturity was due to nutrient dilution as the biomass increased, while an increase could be a sign that these nutrients continued accumulating throughout growth. The response to developmental stages was consistent with Experiment 1 for K, Ca, Mg, Fe and Cu.

At anthesis, herbage nutrient concentration (%; mg/kg) differed ($P \leq 0.02$) among the genotypes for all nutrients, except Mg and S (Figure 5.3, 5.5). For example, PFR-3019 had higher concentrations of N, K, Fe, and Cu, while 'Discovery' had higher N, K, Ca, Cu, and Mn.

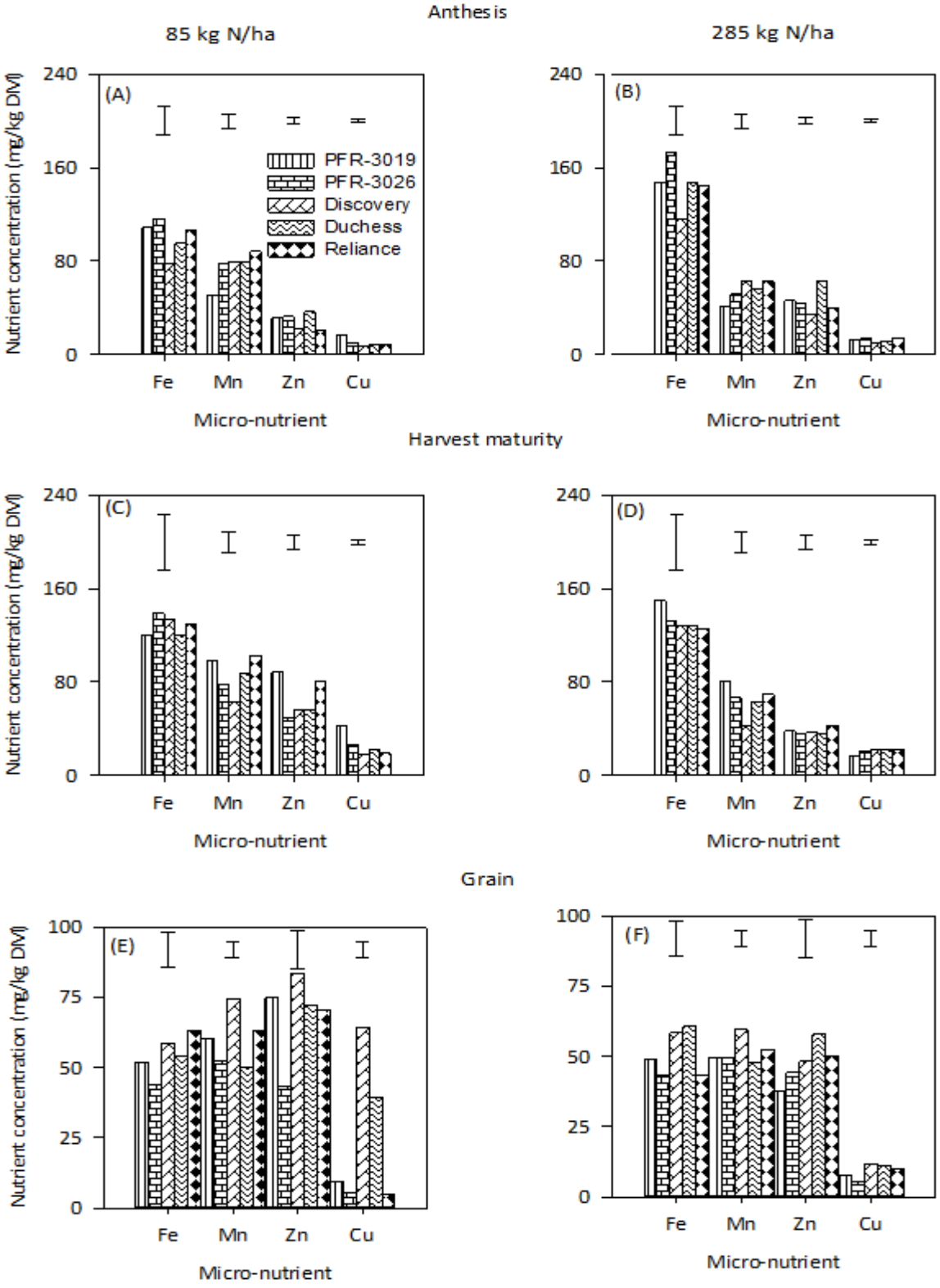

Figure 5.5: Weighted shoot (A—D) and grain (E—F) micro-nutrient concentration (%) at anthesis (A and B) and harvest maturity (C and D) and for five wheat genotypes grown at low (85 kg; A, C, E) or optimum (285 kg; B, D, F) N fertiliser supply (kg/ha) at Lincoln, Canterbury in 2017-18 season. Vertical bars are the leaf significant differences ($LSD_{5\%}$) for the genotypes at each nitrogen levels

At anthesis, herbage nutrient concentration increased ($P < 0.001$) with increasing N fertiliser supply for all nutrients (Figure 5.3, 5.5), except Mn which decreased ($P < 0.001$) from 75.2±3.48 mg/kg DM for the low N crops to 55.0 mg/kg DM when 285 kg N was applied. Overall, the herbage nutrient concentrations in whole shoots at anthesis were within the ranges of 1.0—4.0% for N and K, 0.10-0.40% for P, S, Ca and Mg (Figure 5.3 A, B), and 10-60 mg/kg DM for Mn, Zn and Cu, and 120±11.2 mg/kg for Fe (Figure 5.5 A, B). Nutrients concentrations were within or above the reported threshold concentrations for optimal growth (Reuter et al. 1997).

At harvest maturity, nutrient concentration differed ($P \leq 0.01$) among genotypes for N, K, Mn, Zn and Cu only (Figure 5.3, 5.5). 'Discovery' had higher concentrations of K, Mn, Cu and Zn, while PFR-3019 had higher concentrations of N. Furthermore, herbage nutrient concentrations at harvest maturity increased ($P < 0.001$) with increasing N fertiliser supply for K, S and Ca, and decreased ($P \leq 0.04$) for Mn, Zn and Cu. For example, K concentration increased from 1.15±0.10% for the low N crops to 1.68% when 285 kg N/ha was applied, while Zn concentration decreased from 66.0±3.64 mg/kg DM for the 85 kg N/ha to 38.0 mg/kg DM when 285 kg N/ha was applied. Concentrations of N, P, Mg and Fe were unaffected ($P \geq 0.25$) by N supply, at an average of 1.10±0.05%, 0.25±0.01%, 0.12±0.01% and 132±14.2 mg/kg DM, respectively. The lack of a response to N supply for these elements was because the proportion of nutrient accumulation and shoot biomass accumulation for low N and optimum fertiliser N fertiliser supply, were similar at 33%. Overall nutrient concentrations at harvest maturity were in the range 1.0—2.0% for N and K, and 0.10—0.20% for P, S, Ca and Mg (Figure 5.3 C, D), and 20-85 mg/ kg DM for Mn, Zn and Cu, and 130±11.2 for Fe (Figure 5.5 A, B).

5.3.5.3 Grain nutrient concentration (%; mg/kg)

Grain nutrient concentration was affected ($P \leq 0.02$) by the interaction between N fertiliser supply and genotype for S and Cu concentrations only. The grain S concentration for 'Discovery' and 'Duchess' increased ($P = 0.02$) with increasing N fertiliser supply (Figure 5.3 E, F), while grain Cu concentration for the same genotypes decreased ($P < 0.001$) with increasing N fertiliser supply (Figure 5.5 E, F). However, S and Cu concentrations were unaffected by increasing N fertiliser supply for the other genotypes.

The nutrient concentration for the grain differed among the genotypes for all nutrients except Ca, Mg, and Fe (Figure 5.3, 5.5). 'Discovery' had higher K, Mn and Cu, while 'Duchess' had higher K and S. PFR-3026 had lower P and Zn. Nutrient concentration response to N fertiliser supply was variable: increased ($P \leq 0.02$) for P, S and Zn and decreased ($P < 0.001$) for K, Mn and Cu concentration with increasing N fertiliser supply, while the other nutrients were unaffected ($P \geq 0.36$) by increasing N fertiliser supply.

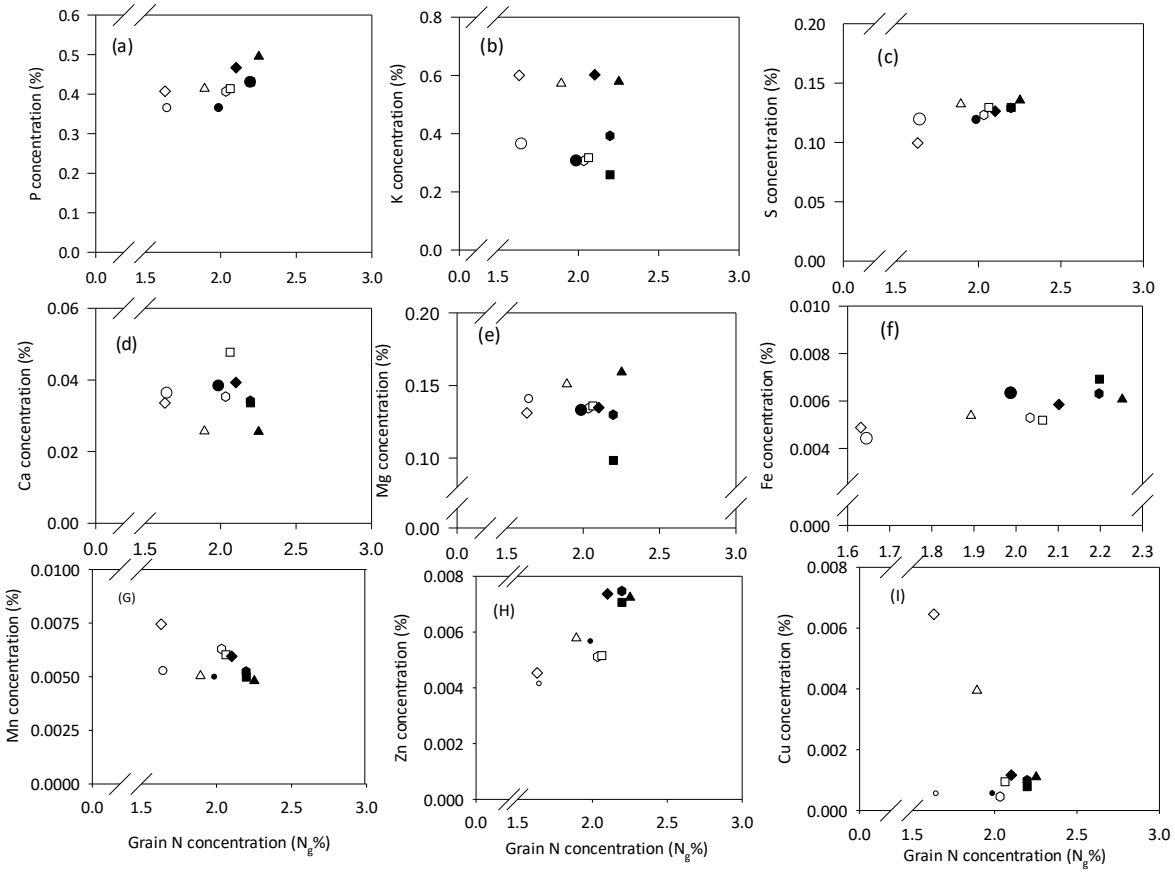

Figure 5.6: Grain nutrient against grain nitrogen concentration (N_g%) for six wheat genotypes (● PFR-2021, ▼ PFR-3019, ■ PFR-3026, ◆ Discovery, ▲Duchess and ⬣ Reliance) grown at low (open symbols) and optimum (closed symbols) N fertiliser supply at Lincoln, New Zealand in 2017-2018 growing season.

The relationships between N_g% and other mineral concentrations (Figure 5.6) was positive and close ($R^2 \geq 0.44$) for P, S and Zn and negative and poor ($R^2 \geq 0.25$) for K, Mn and Cu. There was no relationship ($R^2 \geq 0.02$) between N_g% and Ca and Mg concentration. The relationship between N_g% and K, S and Zn concentrations were consistent with Experiment 1, while P decreased with increasing N_g% in Experiment 1.

5.3.5.4 Total nutrient accumulation (mg/tube)

Overall nutrient accumulation increased with developmental stage for all nutrients except for N, K and S which did not change and were on average: 293±18.2, 434±26.4 and 34.5±2.31 mg per tube, respectively. This represented 88±6.16%, 94±5.91% and 84±5.20% for total N, K and S accumulated at anthesis. However, P increased from 52.0 mg/tube at anthesis (Appendix 5.2) to 69.9 mg at harvest maturity (Figure 5.7), while Fe increased from 2.0 mg/tube at anthesis to 3.85 mg/tube at harvest maturity (Figure 5.8).

At antheis, there was an interaction ($P < 0.001$) between N fertiliser supply and genotype for nutrient accumulation (Appendix 5.1), as there were no differences among the genotypes at low N fertiliser supply. In contrast, nutrient accumulation differed ($P \leq 0.002$) among the genotypes when 285 kg N/ha was applied, except for Cu and Fe. For example, total P was higher for 'Discovery' at 126 mg/tube, compared with an average of 67±5.1 mg for PFR-3019 and PFR-3026, while total Mn was also higher for 'Discovery' at 1.76±0.10 mg/tube compared with 0.61 mg for PFR-3019. This result mirrored the shoot biomass pattern (Table 5.1).

At harvest maturity, nutrient accumulation differed ($P < 0.001$) among the genotypes for all nutrients, except Fe (Figure 5.7; 5.8). 'Discovery' had the highest nutrient yields for all elements, while 'Duchess' was high in P, S, Mg and Cu. 'Reliance' had the lowest P, K, S, Mg and Cu, while PFR-3019 had lower Mg, Mn and Zn. Nutrient accumulation was related to the biomass yield, as 'Discovery' had the highest yield and 'Reliance' had the lowest biomass yields (Table 5.1). Nutrient accumulation increased ($P < 0.001$) with N supply, for example P increased from 31.9 mg/tube for the 85 kg N/ha crops to 99.4 mg when 285 kg N/ha was applied.

The proportion of total nutrient at anthesis (Appendix 5.1) compared with the total at harvest maturity (Figure 5.7), was on average: 88±6.16% for N, 69±4.91% for P, 94±5.91% for K, 84±5.20% for S, 77±6.70% for Ca, 51±4.30% for Mg, 59±6.83% for Fe, 47±4.10% for Mn, 45±1.90% for Zn and 31±3.50% for Cu. The proportions were affected by N supply for all nutrients except Ca, Fe and Cu. In all cases proportions increased with increasing N fertiliser supply, except for Mn which decreased with increasing N fertiliser supply from 52±6.46% for the 85 kg N/ha crops to 42% when 285 kg N/ha was applied. The proportion of nutrients

also differed among the genotypes for S, Ca, Fe, Zn and Cu only. For example, 'Duchess' had higher S and Zn, while PFR-3019 had higher values for Fe and Cu.

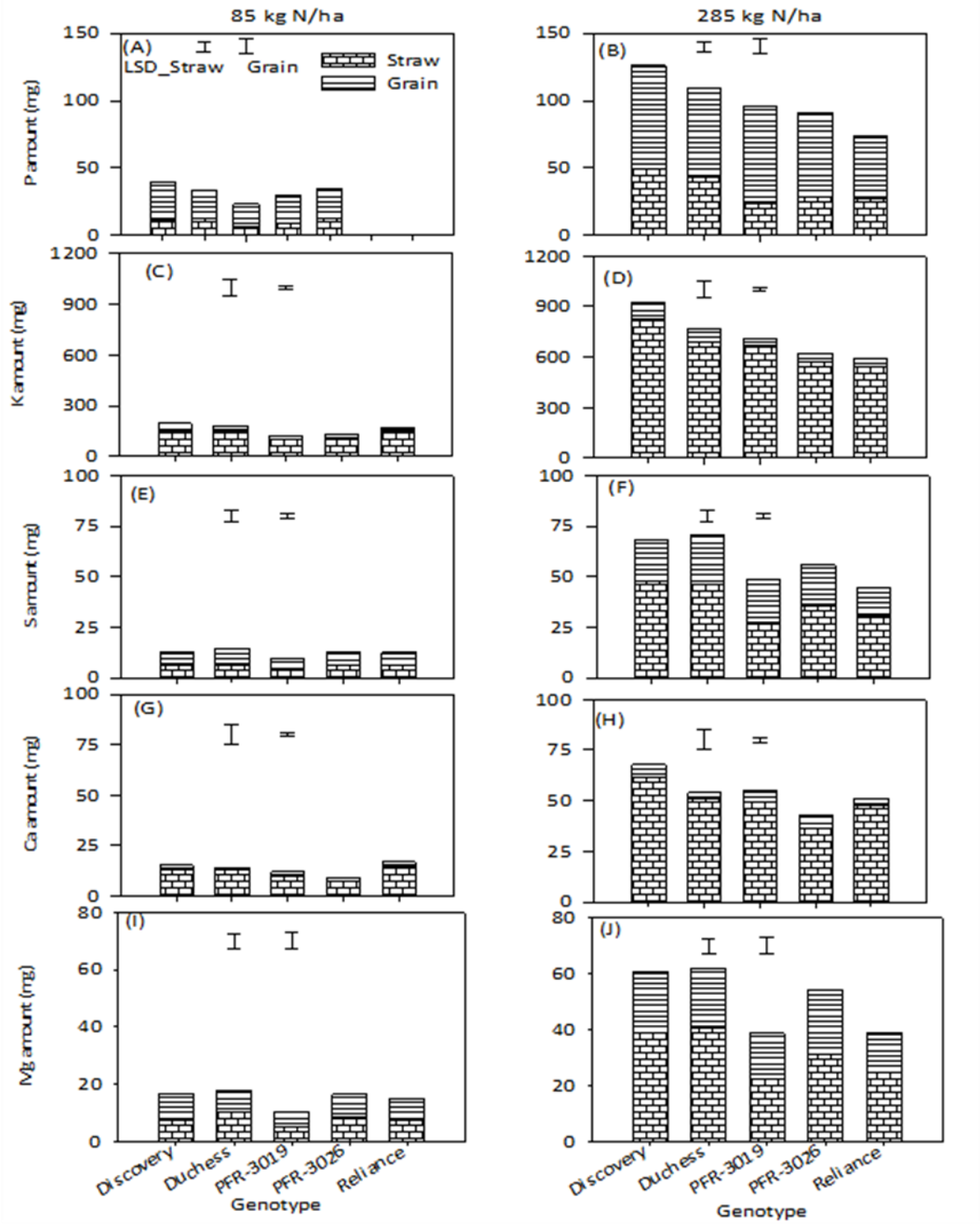

Figure 5.7: Total macro-nutrient accumulation (mg/ tube) at harvest maturity for five wheat genotypes grown at low (85 kg/ha; A, C, E, G, I) and optimum (285 kg/ha; B, D, F, H, J) nitrogen (N) in a Glasshouse at Lincoln, Canterbury, New Zealand in 2018-2019 season. Vertical line are the least significant differences (LSD5%) for straw and grain amounts.

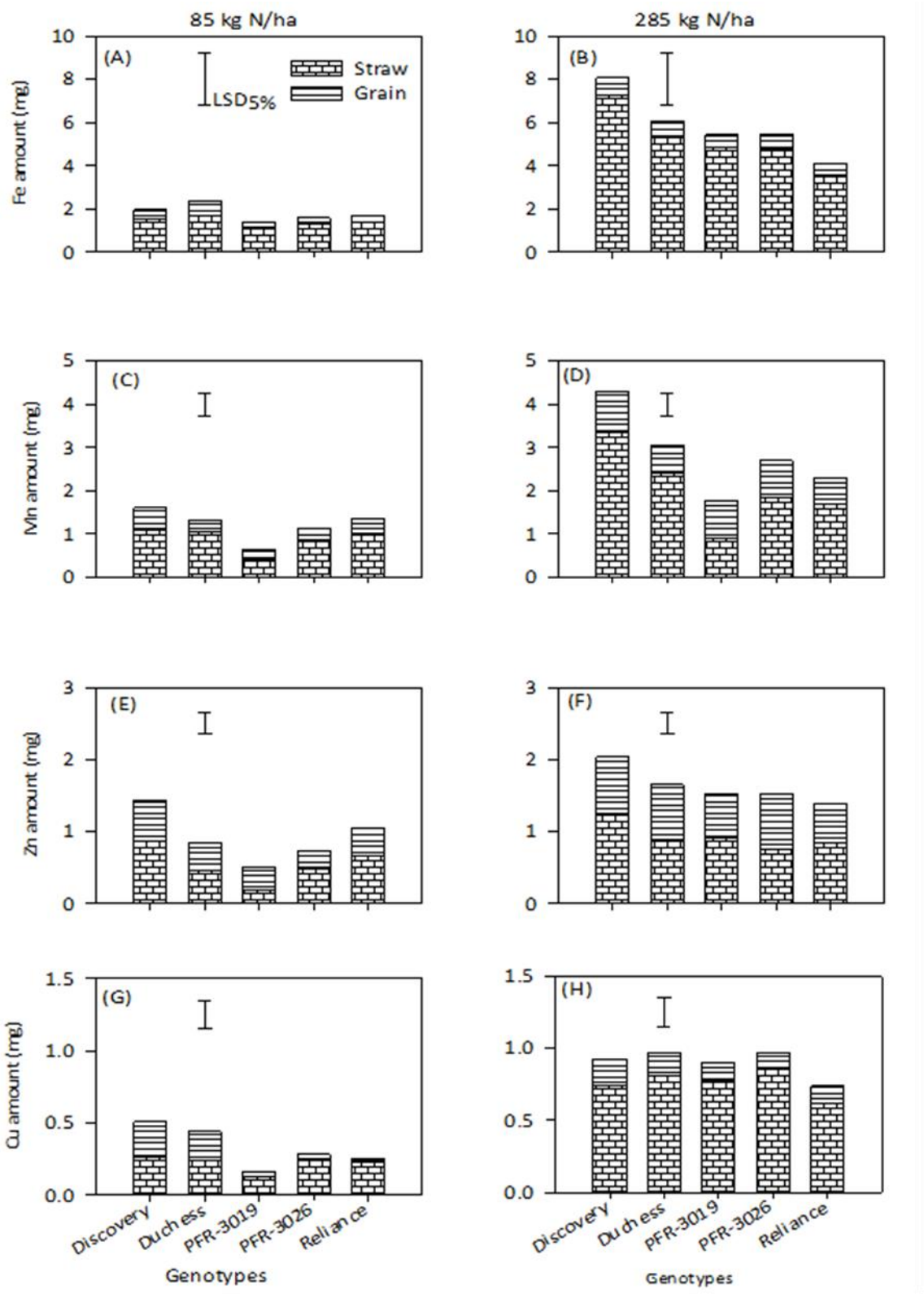

Figure 5.8: Total micro-nutrient accumulation (mg/ tube) at harvest maturity for five wheat genotypes grown at low (85 kg/ha; A, C, E, G) and optimum (285 kg/ha; B, D, F, H) nitrogen (N) in a Glasshouse at Lincoln, Canterbury, New Zealand in 2018-2019 season. Vertical line are the least significant differences (LSD5%) for straw and grain amounts.

Overall, the proportion of Mg, Fe, Mn and Zn was consistent with biomass yield (50±0.03%) (Section 5.3.1), while that for Cu was lower, and the rest of the nutrient elements accumulated faster than biomass.

5.3.5.5 Grain nutrient accumulation (mg/tube)

There was only an interaction ($P = 0.005$) between N fertiliser supply and genotype for Cu. This was because grain Cu accumulation was unaffected by N supply for all the genotypes, except for 'Discovery', for which Cu accumulation decreased from 0.43 mg/tube for the 85 kg N/ha to 0.19 mg when 285 kg N/ha was applied. Grain nutrient accumulation differed among the genotypes for all nutrient elements, except for Ca, Mg and Zn (Figure 5.4; 5.7), because their concentrations were not different among genotypes. Grain nutrient accumulation increased ($P < 0.001$) with increasing N fertiliser supply for all nutrients except Cu ($P = 0.82$), which was 0.14±0.03 mg. The differences in grain nutrient accumulation among the genotypes was a reflection of their concentrations and grain yield.

5.3.6 Nitrogen to nutrient ratios (e.g. N: P or N: Cu)

The responses of N: mineral nutrient ratios across developmental stages were inconsistent (Figure 5.9, 5.10): increased ($P < 0.001$) for N: P, N: K, N: S and N: Ca; decreased ($P \leq 0.008$) for N: Cu and N: Mn and was unaffected ($P \geq 0.22$) for N: Mg, N: Zn, and N: Fe. For example the N: P ratio increased from 5.30 at anthesis to 7.11 at harvest maturity, while N: Cu decreased ($P < 0.001$) from 1,468 at anthesis to 805 at harvest maturity. N: Fe ratio was 131±7.6 at anthesis and harvest maturity.

These responses were consistent with Experiment 1 (Section 4.4.4) for N: K and N: Ca which increased with developmental stage, while N: Mn was unaffected, and N: Cu decreased. These responses were associated with the nutrient accumulation dynamics. Those elements taken up earlier in the season (e.g. K or Ca) (Section 5.3.4.4) were higher, than those taken up later in the season (e.g. Mn or Cu).

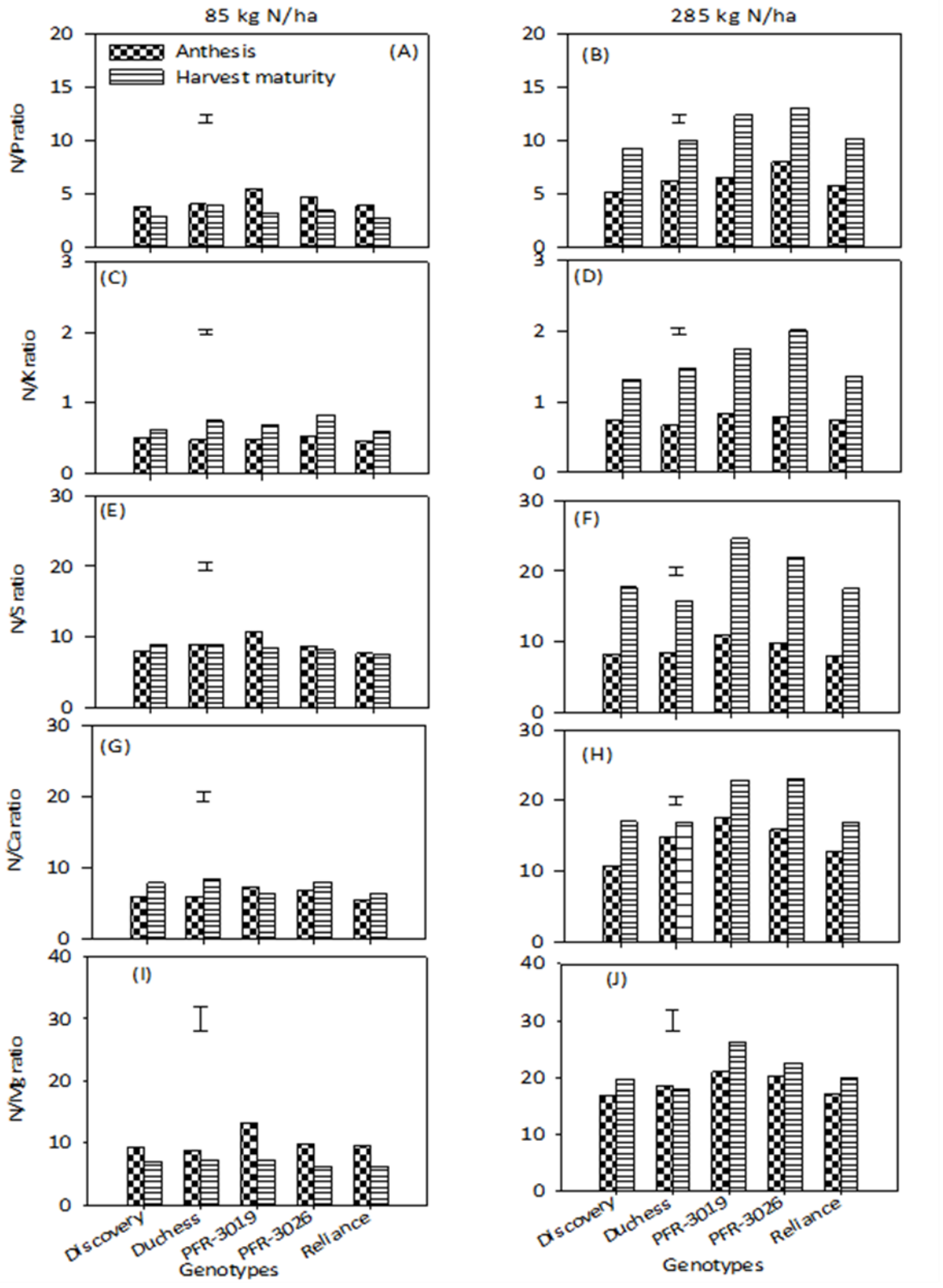

Figure 5.9: Plant nitrogen (N) to macro-nutrient ratios at anthesis and harvest maturity for five wheat genotypes grown at low (85 kg/ha; A, C, E, G, I) and optimum (285 kg/ha; B, D, F, H, J) N fertiliser supply in a Glasshouse at Lincoln, Canterbury, New Zealand in 2018-2019 season. Vertical bars are the least significant differences ($LSD_{5\%}$) for the interaction between harvest and genotype.

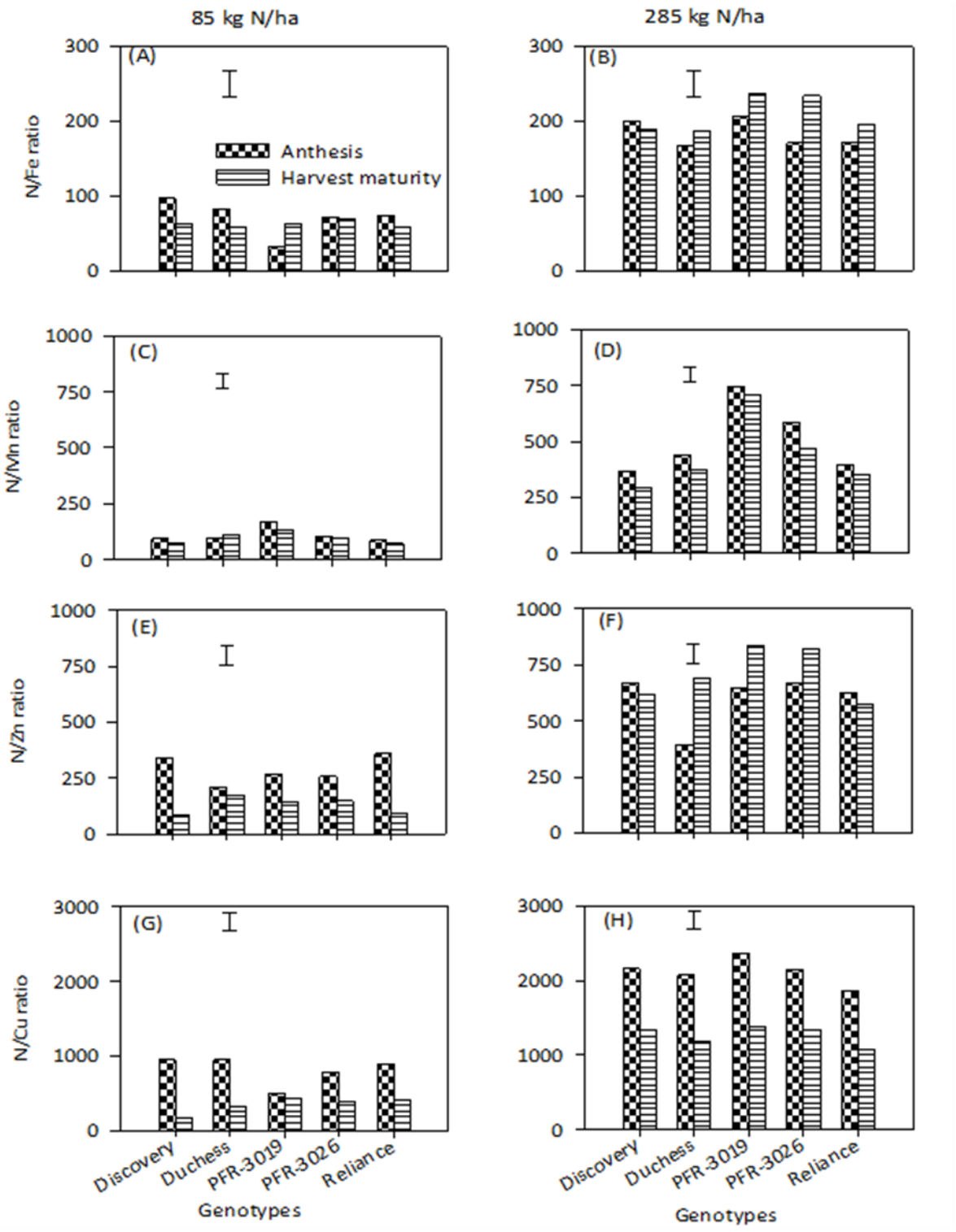

Figure 5.10: Plant nitrogen (N) to micro-nutrient ratios at anthesis and harvest maturity for five wheat genotypes grown at low (85 kg/ha; A, C, E, G) and optimum (285 kg/ha; B, D, F, H) fertiliser N fertiliser supply in a Glasshouse at Lincoln, Canterbury, New Zealand in 2018-2019 season. Vertical bars are the least significant differences ($LSD_{5\%}$) for the interaction between harvest and genotype.

The N: mineral nutrient ratios differed ($P < 0.001$) among the genotypes (Figure 5.9; 5.10) for all nutrients, except N: Fe and N: Cu at anthesis and harvest maturity. At both harvests, PFR-3019 had higher N: S, N: Ca, N: Mg and N: Mn, while PFR-3026 had high N: P and N: K ratios compared with the other genotypes. In contrast, 'Discovery' had the lowest N: P, N: S and N: Ca ratios, while 'Duchess' was low in N: K and N: Zn. This was because PFR-3019 had the lowest N accumulation at anthesis and harvest maturity (Figure 5.4) and lower values for the respective nutrients (Appendix 5.2, Figure 5.7). In contrast, 'Discovery' had the highest N yield at anthesis and harvest maturity (Figure 5.4) and highest respective nutrients (Appendix 5.2, Figure 5.7, 5.8). PFR-3026 had high N: P and N: K ratios compared with the other genotypes, because it had the moderate to high N and low P and K amounts (Figure 5.4; 5.7, Appendix 5.1).

All ratios increased with N supply at anthesis (except for S) and harvest maturity. The N: S ratio was 7.95±0.08 at anthesis and harvest maturity. The N: K ratios were the closest to a 1:1 relationship (Figure 5.9) by weight, while Mg and Ca were most scattered at anthesis and harvest maturity.

Grain N: nutrient ratio differed among the genotypes for N: P, N: K, N: S and N: Mn. 'Discovery' had a lower N: P ratio at 4.20±0.25 compared with 4.94 the other genotypes and 'Duchess' had lower N: S of 11.9±0.46 compared with 16.2 for the other genotype. 'Duchess' and 'Discovery' had higher N: Mn ratio at 1,033±88.4 compared with 706±88.4 for the other genotypes.

Grain N: nutrient ratios increased with increasing N fertiliser supply for N: Fe, N: Mn and N: Zn only. For example, N: Mn increased from 314: 1 for the 85 kg N/ha crops to 417: 1 when 285 kg N/ha was applied.

5.3.7 Nutrient remobilisation (NuR) and post-anthesis nutrient uptake (PANuU)

Nutrient remobilisation (NuR) was determined from data in Appendix 5.1 and the straw component of Figures 5.7 and 5.8. The NuR differed among the genotypes for P, K, S and Zn only. 'Discovery' remobilised 41.8±5.20 mg P/tube compared with 26.5 mg P/tube for the other genotypes, while PFR-3019 remobilised lower S (7.65 mg/tube) and K (0 mg) compared with 13.9 mg/tube and 54.8±40.7 mg/tube for the other genotypes, respectively. 'Duchess' remobilised more Zn at 0.25±0.12 mg/tube compared with 0 mg for the other

genotypes. The NuR increased with N supply for P, K and S only. For example, P remobilisation was 5.40 mg/tube for the low N fertiliser supply compared with 53.6 mg when 285 kg N/ha was applied.

Post-anthesis nutrient uptake (PANuU; Equation 3.4) was determined from data in Appendix 5.1 and Figures 5.4, 5.7 and 5.8. The PANuU differed among the genotypes for Ca and Mg; PFR-3019 had higher PANuU for Ca at 17.0 mg compared with 6.83±3.60 mg for the other genotypes, while 'Duchess' had higher PANuU for Mg at 20.5 mg compared with 12.4±4.06 mg for the other genotypes. The PANuU differed among the N fertiliser rates for all nutrients except for P, K and Zn. PANuU was negative for N at both N fertiliser rates.

5.4 Discussion

The objective of this study was to determine the effects of genotype, N fertiliser supply and their interaction on CHI, NHI, NuHIs and NUE for six spring sown wheat genotypes, and to confirm genotype ranking of results from Experiment 1 (Chapters 3 and 4). Results show that all the key traits under consideration: CHI, NHI and NUE differed among the genotypes, and were consistent for both experiments. For example, 'Duchess' had the lowest CHI in both experiments, while 'Discovery' and 'Duchess' had the lowest NHIs and 'Discovery' had the highest NUE in both experiments. Furthermore, NuHIs differed among genotypes for some elements; however, these were not consistent between the experiments. The differences in grain yield among the genotypes in both experiments showed genetic differences existed, which can be further explored in future breeding programmes. In particular, under optimum N conditions, 'Discovery' consistently produced a higher grain yield than 'Reliance'. The interaction between N fertiliser supply and genotype for the grain yield in Experiment 1 was highlighted by the two high yielding genotypes ('Discovery' and PFR-2021). They both produced their highest grain yield at optimum N fertiliser supply, but at low N fertiliser supply, 'Discovery' produced a higher yield than PFR-2021. This result was not repeated in Experiment 2 because PFR-2021 was discarded due to early flowering and bird damage. Nutrient accumulation was split evenly pre- and post-anthesis for Mg, Fe and Zn in both experiments, while 78—100% of Ca and K was accumulated pre—anthesis, and ≥ 68% of Cu was accumulated post—anthesis. The relationship between N_g% vs. S and Zn concentrations were close and positive ($R^2 \geq 0.44$), while that to K concentration was negative in both experiments. The relationship between N_g% and P concentration was

inconsistent, poor (R^2 = 0.006) and negative in Experiment 1 and positive and close (R^2 = 0.45) in Experiment 2.

5.4.1 Crop harvest index (CHI)

The low CHI for 'Duchess' in both experiments was attributed to a combination of low grain yield and high total shoot biomass (Table 3.5; 5.1). These results suggest that 'Duchess' did not remobilise the extra biomass into the grain, which is reflected in its low TGW in both experiments (Figure 3.4; Table 5.2) and high screenings (Table 3.6) in Experiment 1. There was no relationship between grain yield and CHI in Experiment 1 and 2 (Tables 3.4, 3.5, 5.1), as the highest yielding genotype 'Discovery' had low and moderate CHI, in Experiment 1 and 2, respectively. Furthermore, the lowest yielding genotype, 'Reliance' had high CHI in both experiments, while 'Duchess' had the same grain yield as 'Reliance' but lower CHI in both experiments. Average CHIs of 0.55±0.01 reported for Experiment 1 and 0.42±0.03 for Experiment 2 (Table 3.4, 5.1), were consistent with the range of 0.40—0.60 reported for modern, elite genotypes (Le Gouis et al. 2000; Barraclough et al. 2010; Gaju et al. 2011; Dai et al. 2016). These values are also consistent with the maximum calculated CHI of 0.40-0.50 for short-strawed, lodging-resistant wheat crops (Austin et al. 1980; Austin 1999; Berry et al. 2007; Senapati & Semenov 2019). The lower CHI in Experiment 2 could be attributed to the high temperature episodes near anthesis (Appendix 5.3), which has been reported to reduce grain density (Wheeler et al. 1996; Ferris et al. 1998), resulting in lower grain yields, and the subsequent lower CHI. The mean temperatures around anthesis (November 2018) in the Experiment 2 was 19.8°C (30.5°C maximum and 16.0°C minimum) compared with mean of 18.0 (27.6°C maximum and 6.3°C minimum) for Experiment 1 (Figure 3.1). This would explain the low grain density in Table 5.2. Furthermore, higher maximum temperatures above 30°C were also recorded during grain filling period in December 2018 and January 2019. Unlike in Experiment 1 (Section 3.9.1), CHI in Experiment 2 decreased with increased N fertiliser supply. This could be due to severe N stress experienced by the crops as there is no N mineralisation under the growing media used with limited starting basal N of <1 mg/L of nitrate-N and ammonium-N (Section 5.2.1), resulting in limited number of grain (Table 5.2). This severe N stress limited straw yield for the low N crops more than the grain (Table 5.1), hence the difference in CHI between fertiliser treatments.

Higher AGB and grain yield for 'Discovery', compared with 'Reliance' in both experiments was associated with its early LAER and high leaf area at anthesis (Figures 3.5; 5.1). This allowed 'Discovery' to attain high maximum GLAI, and a longer LAD. In contrast, the low green leaf area for 'Reliance' meant a shorter period of canopy closure and thus less captured IPAR, leading to the low yields. Consistent with Experiment 1, the SLN of ≥ 1.37 g N/m^2, was higher than the SLN threshold of 1.1 (0.8—1.30) g N/m^2 (Meinke et al. 1998) and therefore SLN did not affect photosynthetic capacity and hence reduce RUE (Sinclair & Horie 1989) among the genotypes. Thus, the differences in yield were attributed to IPARi in both experiments.

In both experiments, grain yields were more closely related (R^2 = 0. 67—0.90) to the grain density, than TGW (R^2 = 0. 001—0.01). However, in both experiments, when TGW was plotted against grain density (Figure 3.4; 5.2) the highest yielding genotype 'Discovery' was positive and above the regression line. This suggests it had more total carbon in the grains (weight x density) than the average of the other cultivars and was therefore consistently able to produce a higher TGW at the same grain density. The implication is that TGW can be used as a discriminator of total carbon captured and allocated to the grain. Specifically, by definition all genotypes that showed TGW located positively above the regression line (Figure 3.4; 5.2), produced more grain yield. The corollary is that the TGW for the low yielding genotypes, e.g. 'Reliance' and PFR-3019, were always below the regression line. This shows they did not have access to the same amount of assimilate to remobilise or allocate to the grain, and hence their low TGW and grain yield relative to grain number. This combination of low TGW, low grain yield but high AGB led to low CHI for 'Duchess'. These results further confirm that the differences in relationship between TGW and grain density can be used to explain grain yield differences. The importance of the trade-off between TGW and grain density is not purely speculative as described in Section 2.2 and 3.9.1.1.

Previous researchers have indicated the linearity of the relationship between TGW and grain density as an indicator of mutual compensation in yield components (Peltonen-Sainio et al. 2007). The responsiveness of grain density was emphasised when comparing the role of yield components in relatively low yielding environments, while the role of TGW in yield determination increased among higher yield groups (*Loc. cit*), e.g. 'Discovery' vs. 'Reliance' in our study. Peltonen-Sainio et al. (2007) suggested that selection for any individual yield

component is therefore unlikely to result in increased grain yields. However, analysis of the residuals would appear to offer a universal indicator of total carbon allocated to grain with positive residuals highlighting genotypes that had either more total carbon available and allocated the same proportion. The consistency of these results between experiments suggests it may be an indicator of the vegetative differences observed amongst genotypes that resulted in more total carbon available for 'Discovery' which led to its greater grain yields but small, lower (6.70%; Experiment 1) and no difference (Experiment 2) in CHI. This means 'Discovery' and 'Reliance' allocated the same proportion of carbon to the grain. This result is important because it contradicts the now well documented (e.g., Austin et al., 1989) genetic gains in wheat yields that have been accompanied by increased CHI, but little discernible trend in AGB. Austin (1999), reported that there was only limited scope for a genetic increase in biomass while the pool of variation available to breeders is confined to the *Triticineae*. He suggested that photosynthetic characteristics can be improved by genetic 'engineering', to raise the biomass ceiling and hence yield. However, the results from the current studies show that there is still scope within the genotypes tested to increase total AGB by focusing on the vegetative traits that increased IPARi. In a breeding situation, the relationship between TGW and number of grains may be a useful indirect method for such selections to be made. The impact of such selection, coupled with potential yield increases from elevated-atmospheric CO_2 concentrations, means further yield increases may be possible without genetic engineering. The effects of elevated CO_2 for the highest yielding genotype 'Discovery' will be examined in Chapter 6.

5.4.2 Nitrogen harvest index (NHI)

For both experiments, NHI was lower for 'Discovery' and 'Duchess' (Figure 3.9; 5.4 C, D), due to their higher total shoot N yield compared with the other genotypes, as there were no genotypic differences in N_g. Most of the differences in NHI were explained by shoot N yield. Average NHI of 0.79—0.86 in Experiment 1 and 0.72—0.78 in Experiment 2, were within the range of 0.70—0.90 reported for modern spring and winter wheat genotypes (Le Gouis et al. 2000; Andersson & Johansson 2006; Gaju et al. 2011; Gorjanović et al. 2011; Pask et al. 2012; Belete et al. 2018).

The N_g% in the two experiments ranged from 1.85% to 2.30% (in DM), increased with increasing N supply and differed among genotypes as reported previously (Le Gouis et al.

2000; Barraclough et al. 2010; Gaju et al. 2011; Pask et al. 2012). This translated to 10.6—13.1% GPC, thus the upper limit was within the standard quality requirements for milling wheat in New Zealand, of ≥11.4% GPC (Grama et al. 1987; Reddecliffe et al. 2000), based on a conversion factor of 5.7 (Lopez-Bellido et al. 2004). Specifically, wheat genotypes in New Zealand are classified as 'premium' (e.g. 'Reliance' and 'Duchess') whose GPC generally sit around 12.0 -13.5% or 'milling'/ 'medium' genotypes (e.g. 'Discovery') which tend to be lower at around 11.0-12.5% (Munro, Catherine., pers.comms). However, these ranges depend on the genotype, environment and crop management (G*E*M) as reported in a recent review (Hatfield & Walthall 2015). At the optimum N fertiliser supply, all genotypes attained the minimum milling quality requirements in both experiments. However, at low N, only PFR-3019, PFR-2021 and 'Reliance' attained the milling quality requirements. Differences in grain yield reported in both experiments were consistent with those reported for other short-strawed genotypes (Austin et al. 1980; Austin 1999; Barraclough et al. 2010) and resulted in higher N_g% and GPC for the low yielding 'Reliance' compared with high yielding 'Discovery', affirming their current classification in New Zealand as premium and medium quality wheats, respectively (FAR 2019).

The ranking of N_g% for all genotypes in both experiments, followed the well-known inverse relationship between yield and N_g% (Drewitt 1979; Austin et al. 1980; Cox et al. 1985; Guarda et al. 2004; Triboi et al. 2006), except PRF-2021 in Experiment 1. However, as the relative variation in grain yield was greater than the variation in N_g%, the total amount of N in the grain was greater for the higher yielding genotypes, similar to previous reports (Austin et al. 1980), and hence the higher NUE (Section 3.8.4; 5.4.3). The negative relationship between N_g% and TGW reported in both experiments was consistent with previous reports (Drewitt 1979) and attributed to the dilution of the limited N_g by the increasing carbohydrate component of the grain, due to disproportionately more structural protein being laid down early in the grain-filling process (Martre et al. 2003), a period when accumulation is sink regulated. When the objective is to grow for high GPC, then 'Reliance' would be the most appropriate choice, while for high yield, but moderate GPC, 'Discovery' would be the appropriate genotype. The current situation in New Zealand, is to blend grains from different genotypes, producing 'grist' (the industry term for a blend) with the required GPC (Munro, Catherine., pers.comms), using both 'premium' and 'medium' quality wheat.

The farmers question is which is most profitable and that depends on the price relationships between yield and protein offered by the mills. However, in Experiment 1, PFR-2021 responded differently to the established rules on N_g% vs. grain yield or plant height (Section 3.9.2). This was attributed to its higher PANU (Section 3.8.1.1).

5.4.3 Nitrogen use efficiency (NUE)

In both experiments, the differences in grain yield resulted in higher NUE for 'Discovery' compared with 'Reliance'. Results show that the differences in NUE among the genotypes were marginal in both experiments, particularly at low N fertiliser supply. This was mainly attributed to the marginal, no grain yield differences at low N fertiliser supply (Tables 3.4, 5.1) and similar N_g yield across the genotypes for both experiments (Sections 3.7.6.3; 5.3.3.4). The range of NUE reported in both experiments of 30.0—77.0 (Tables 3.10, 5.3) was comparable to the 20.0—65.0 reported for modern spring wheat (Kubota et al. 2018).

The variation in NUE in the current studies was explained (R^2 = 0.45—0.54) by NupE in Experiment 1 for both N fertiliser rates and NutE (R^2 = 0.25) at low N fertiliser supply. In contrast, in Experiment 2, NUE was explained by NutE (R^2 = 0.50—0.60) for both N fertiliser rates, and NupE (R^2 = 0.61) at optimum N fertiliser supply. This could be a reflection of the environment in which the crops were grown. Nitrogen was in a readily available form in Experiment 2 (as applied N fertiliser) compared with soil N in Experiment 1, which is predominantly mineralised during the season. There is a risk that this N may be lost (e.g. leaching) or may not be mineralised at the rate of crop demand; hence, the uptake efficiency becomes important. Previous worldwide investigations examining historic sets of genotypes have similarly shown conflicting results. The NupE accounted for a greater proportion of genetic variation in NUE at low than optimum N fertiliser supply (Ortiz-Monasterio et al. 1997; Le Gouis et al. 2000; Muurinen et al. 2006; Gaju et al. 2011), while others reported greater association to NutE (Foulkes et al. 1998; Brancourt-Hulmel et al. 2003). The NutE has been reported to explain genetic variation in NUE with increasing N supply in spring wheat (Ortiz-Monasterio et al. 1997); and the limited evidence from a small number of genotypes tested in this study, would support this observation for the glasshouse, but not field experiment. In contrast, Dhugga and Waines (1989) reported that NupE was the more important component of NUE at both low and optimum N conditions. In the current study, the reported NupE of 0.68—1.50 and NutE of 34.0—52.1 are consistent

with the ranges reported by Gaju et al. (2011) and Pask et al (2012). The current results are sensible as the genetic pool is limited to modern genotypes (Appendix 1.1), compared with the generation interval of previous reports (*Loc. cit)*. Furthermore, NupE at higher N and NutE at low N (Bertin & Gallais 2000) have accounted for NUE in other crops, e.g. maize. Thus, the differences in NUE or its components among the genotypes reported here are important for future breeding if they are used as parents.

The apparent N fertiliser recovery (AFR) rate of 45% (Section 3.8.1) was within the estimated 30—50% reported for winter wheat crops (Raun et al. 2002), and close to the *ca.* 50—60% reported for high yielding winter wheat crop in NW Europe (Austin et al. 1993; Sylvester-Bradley et al. 1997). This result suggests comparable AFR for spring and winter wheat. However, these reported AFR values meant that 55% of the applied N in Experiment 1 was unaccounted for, and exposed to losses, e.g. through leaching or volatilization (Raun & Johnson 1999), which affect the environment. Therefore there is an increasing emphasis globally to breed wheat genotypes with potential for improved NUE (Hirel et al. 2007; Foulkes et al. 2009a), thus reducing excessive synthetic N fertiliser inputs while maintaining acceptable grain yield. Breeding for NUE has to balance with GPC, as there is an inverse relationship between grain yield and GPC (Section 5.4.2), therefore crop management should also be used to increase NUE, e.g. fertiliser supply and management.

5.4.4 Nutrient harvest indices for other nutrients (NuHI)

The NuHIs were inconsistent between the experiments, particularly with reference to relationships with genotypes, except for SHI which was high for PFR-3019 and ZnHI, which did not differ among the genotypes in both experiments. For other elements, inconsistent results were observed between the experiments, e.g. KHI was highest for 'Discovery' in the Experiment 2, but lowest in Experiment 1. These contrasting relationships were also observed for the other elements and genotype combinations. This suggests that the growing environment had an impact on partitioning of nutrient, thus NuHIs. However, PHI, MnHI and ZnHI were unaffected by N supply in both experiments.

The NuHIs differed widely between nutrients, from lows of ≤ 0.30 for Ca, Cu, Fe and K to higher values of ≥0.60 for N, Mg and P, and were consistent for both experiments (Figure 4.4; Table 4.4, Appendix 5.2), except for Mn that was moderate in the field (0.57) compared

with the glasshouse (0.29). These ranges reflect individual nutrient phloem mobility (Loneragan et al. 1976; Reuter & Robinson 1997), except for K in both the experiments and Mn in Experiment 1. The NuHIs were lower for the phloem immobile nutrients such as Ca and Fe compared with phloem mobile nutrients such as N and P and intermediate for those of variable phloem mobility e.g. S (Table 4.1; 4.2, Appendix 5.2). The low CaHI and KHI have been reported in other cereals, such as maize (Karlen et al. 1988) and grain sorghum (Hocking 1993). The low translocation of K into the grain in Experiment 2 that was protected from rainfall and where there was no overhead irrigation, contradicts previous reports on foliage K losses through leaching (Tukey 1970). This is consistent with K being less translocated and strongly bound to the structural components of the plant.

The near isometric uptake for Fe, Mg and Zn at anthesis was consistent in both experiments and related to the biomass accumulation at anthesis of 55.0±0.20% for Experiment 1 and 50±0.03% for Experiment 2, compared with biomass at harvest maturity. The lower Cu and higher K and Ca were also consistent between the experiments. Differences in temporal patterns of nutrient accumulation were reported previously for wheat (Hocking 1994; Gaj & Rebarz 2014; Hamnér et al. 2017) and maize (Karlen et al. 1988; Ciampitti et al. 2013; Ciampitti & Vyn 2013). Early uptake is important for Ca and K (Section 4.6.2), as Ca is a constituent of cell walls in leaves, while K is necessary for the development of lignin and cellulose (McLaren & Cameron 1996) which gives strength and vigour to the plants. These results show that nutrient uptake after anthesis was substantial for most nutrients in both experiments, except for Ca and K. This may have implications for nutrient management (Section 4.6.2).

In contrast to the dilution effect, grain S and Zn concentrations showed a clear positive correlation with N_g% in both experiments. Furthermore, grain P and Fe concentration were positively related to N_g% in Experiment 2 but not Experiment 1. Several studies have shown positive effects of N_g% on Zn and Fe (Cakmak et al. 2010) and S (Hamnér et al. 2017) concentration. Nitrogen and S are both constituents of some grain proteins (McLaren & Cameron 1996), and grain proteins have been suggested as a sink for Zn (Section 4.6.3). Therefore, the grain S and Zn concentrations can be increased by enhanced grain protein concentration and thereby the sink strength of the grain for S and Zn. However, the lower positive ($R^2 \leq 0.21$) relationship between N_g% and the other nutrients could be attributed to

the poor relationship between grain yield and grain size (Figure 3.3 D; 5.2 D). In theory, grain size can be expected to affect the grain nutrient concentration through the dilution or concentration mechanisms (Hamnér et al. 2017). However, in our study, the relationships between grain yields and TGW were poor (Figure 3.4; 5.2 C) in both experiments and therefore did not systematically affect grain nutrient concentrations. The implication was that either observed relationships between nutrient concentration and Ng% were independent of concentrations or totals of nutrient per grain (*Loc. cit*) used in the regressions (Figures 4.3; 5.6).

The overall grain nutrient concentrations (Figures 4.1; 4.2; 5.3; 5.5) were within the reported 'adequate' levels (Reuter & Robinson 1997) for human health requirements, except for K (0.36—0.43%) for both experiments and Zn (29.1±1.0 mg/kg) for Experiment 1, which were lower than the 0.50% (Reuter & Robinson 1997) and ≥50 mg/kg (Liu et al. 2014), respectively. Grain Fe concentration of 40.0±1.68 mg/kg in Experiment 1 was borderline, as the recommended is ≥40 mg/kg (Liu et al. 2014). The micro-nutrients discussed here are important for their effects on crop production and their association with substantial global public health problems of micro-nutrient malnutrition (i.e. 'hidden hunger') (Loladze 2002; Myers et al. 2014). The low Fe and Zn concentration in Experiment 1 is of concern and it can be postulated that soil levels were low (not measured), as when adequate nutrients were applied in Experiment 2, the concentration for both elements in the grain were within adequate levels. This suggests that we need to include micro-nutrients in soil test analyses, or apply fertilisers that contain micro-nutrients (e.g. YaraMila products) to avoid deficiencies. The low grain K concentration in both experiments was a surprise as background soil K levels were adequate for both experiment (Table 3.2; Section 5.2.1), and could be a reflection of its low translocations to the grain as discussed Section 4.6.2.

The N: nutrient ratios were consistent for the two experiments for N: K, N: Ca and N: Cu. The N: nutrient ratios are a direct function of N uptake and an inverse function of relevant nutrient uptake (Sadras 2006). Therefore, they tend to increase with developmental stage for the nutrients that were taken up earlier [K, Ca] in the season and were higher at harvest maturity. In contrast, for Cu which was accumulated later in the season, the N: Cu ratio was lower at harvest maturity. These N: nutrient ratios could be used as a surrogate for timing of

fertiliser application as the ratios that are high at maturity, means the relevant nutrients are needed earlier in the season, and vise-versa.

Generally, the monovalent cations (e.g. K^+) are absorbed rapidly (Mengel et al., 2001), whereas the divalent cations (e.g. Ca^{2+}) are absorbed more slowly. Most of the demand for K is during the vegetative growth stages (McLaren & Cameron 1996; Fageria 2015) and plants tend to take up K in excess of their needs. The uptake of K has been reported to be selective and closely related to metabolic activity of plants (Fageria 2015), hence their uptake is during the most active growth stages. The main feature of K is the high rate and efficient means by which it is taken up and translocated throughout the plant (Mengel et al., 2001), which has been attributed to the various K transport systems (e.g. H^+/sucrose cotransport system) occurring in the plasmalemma and tonoplast of plant tissues.

The reported high Ca uptake during the vegetative stages, has been reported previously for wheat (e.g. Hocking, 1994) or grain sorghum (Hocking, 1993) and attributed to the higher Ca concentration in the soil than the efficiency of uptake (Mengel et al., 2001). There is reported antagonism in the uptake of various cations, e.g. Ca and Mg (Grzebisz 2015) often observed as (1) increasing Ca concentration in the rhizosphere solution decreased Mg uptake by roots and its accumulation in shoots and (2) the application of Ca fertilisers can induce Mg deficiency in crops (Hawkesford et al. 2012; White 2012). This could explain why Mg uptake pattern is different from K and Ca, and is only pronounced when both K and Ca uptake have plateaued (post-anthesis).

5.5 Conclusions

Differences in grain yield in both experiments showed genotype differences existed, particularly under optimum N conditions, with ‘Discovery’ consistently producing higher grain yield compared with ‘Reliance’. These results show that there are opportunities to improve grain yield through breeding for faster LAER and higher maximum GLAI, even from a starting soil mineral N of 85 kg N/ha to 1.20 m depth, as the current study. ‘Discovery’ had faster LAER, high maximum GLAI and longer LAD, compared with ‘Reliance’. These results support the hypothesis that active selection for canopy characteristics that confer rapid early leaf growth, particularly during stem extension, can lead to increased grain yields under the conditions examined in this study.

All the key traits under consideration: CHI, NHI and NUE differed among the genotypes, and were consistent for both experiments. 'Duchess' had the lowest CHI and the lowest NHIs, while 'Discovery' had had the lowest NHI and highest NUE in both experiments. Furthermore, NuHIs differed among genotypes for some elements; however, these were not consistent between the experiments. Nutrient accumulation was split evenly pre- and post—anthesis for Mg, Fe, Mn and Zn in both experiments, while 78—100% of Ca and K was accumulated pre—anthesis, and ≥ 68% Cu was accumulated post—anthesis. Furthermore, there was a strong, positive relationship between N_g% and grain S and Zn concentration, but a negative relationship to grain K concentration in both experiments. Additionally, P and Fe concentration also increased positively to N_g% in Experiment 2 only.

The relationship between TGW and grain density, showed that all genotypes with TGW located positively above the regression line produced more grain yield, e.g. 'Discovery' in both experiments. The implication is that TGW can be used as a discriminator of total carbon captured and allocated to the grain. These results further confirm that the relationship between TGW and grain density can be used to explain yield differences, and can be used for future breeding selections.

The CHI (AGB & grain yield) and NuHIs are related to the capture of environmental resources, e.g. CO_2 and light and availability of other macro-nutrients e.g. P and K. These factors are considered in Chapter 6.

Chapter 6: Crop and nutrient harvest indices for wheat grown at low and optimum phosphorus and potassium fertiliser rates at ambient and elevated carbon-dioxide levels

6.1 Introduction

CHI differed among the genotypes in Experiment 1 and 2 (Chapters 3 and 5), and was relatively low for 'Duchess' compared with the other genotypes. However, CHI was unaffected by N fertiliser supply in the Experiment 1. The high straw: grain ratio explained the low CHI for optimum N fertiliser treatments in Experiment 2, as reported previously (McNeal et al. 1971). The average CHIs of 0.55±0.01 for Experiment 1 and 0.42±0.03 for Experiment 2 were consistent with the range of 0.40—0.55 reported for modern wheat genotypes (Angus & Fischer 1991; Le Gouis et al. 2000; Gaju et al. 2011; Dai et al. 2016). The overall, lower CHI in Experiment 2 was attributed to the high temperature episodes near anthesis (Appendix 5.3), which resulted in reduced grain density (Section 5.4.1), and overall grain yield.

NHI differed among the genotypes in Experiment 1 and 2 (Chapters 3 and 5), being lower for 'Discovery' and 'Duchess' compared with the other genotypes (Sections 3.7.6.0 and 5.3.3). Furthermore, the genotype rankings was consistent for both experiments. The NHI decreased with increasing N fertiliser supply for both experiments. The NHI range of 0.72—0.86, across the experiments, was within the 0.70—0.90 reported for modern wheat genotypes (Gaju et al. 2011; Gorjanović et al. 2011; Pask et al. 2012; Belete et al. 2018). The NuHIs differed among the genotypes, except for MgHI, FeHI and ZnHI and decreased with increasing N fertiliser supply except for PHI, MnHI and ZnHI, which were unaffected. The overall NuHI values were consistent across Experiment 1 and 2 (Chapters 4 and 5), except for MgHI and MnHI.

The pattern of nutrient uptake at anthesis was near isometric for Fe, Mg and Zn in Experiments 1 and 2. Hamnér et al. (2017) reported similar results. Furthermore, the lower Cu and higher Ca and K uptake at anthesis were also consistent between the experiments and have been reported previously for wheat (Hocking 1994; Gaj & Rebarz 2014; Hamnér et al. 2017).

Reported effects of eCO_2 on wheat yield and nutrient concentration have been described in Section 2.6. Briefly, eCO_2 stimulates plant growth; resulting in higher yields (Long et al. 2004). In wheat (C3 crops), the growth stimulation is a result of both enhenced photosynthesis and improved water use effeciency (Lawlord & Mitchell 1991). However, present knowledge on the effects of eCO_2 on plant nutrient demand, nutrient concentrations and the grain quality is poor, except for N (Section 2.6). The effects of eCO_2 on CHI are inconsistent. A meta-analysis of data from 29 peer-reviewed literature published between 1980 and 2017 (Broberg et al. 2019) indicated that CHI was unaffected by eCO_2. However, a comprehensive review of 112 experiments by Amthor (2001), found that in 28% of the experiments, CHI declined with eCO_2, while 50% showed an increase with eCO_2. The decrease in CHI at eCO_2 was associated with N stress (Sionit et al. 1981; Goudriaan & de Ruiter 1983), while the positive relationship between CO_2 and CHI was associated with water stress (Gifford 1979; Sionit et al. 1980).

A comprehensive literature search did not find any reports on the effects of eCO_2 on NuHIs, except for the NHI (Hazra et al. 2019) and macro-element HIs for wheat crops grown at optimum nutrient levels (Manderscheid et al. 1995). These authors reported that eCO_2 did not affect the NuHIs for spring-sown wheat, except for NHI (Hazra et al. 2019) which increased with eCO_2 and SHI (Manderscheid et al. 1995), which decreased with eCO_2. As these crops were grown with adequate nutrients, it is unclear how P and K deficiencies would affect NHI and NuHIs at eCO_2 for modern spring sown wheat. Furthermore, there are limited reports on NuHIs for micro-nutrients, dated for spring wheat (Hocking 1994) and for winter wheat (Hamnér et al. 2017), and none under eCO_2, P deficiency or K deficiency or their interactions and therefore this study will be used to determine micro-nutrient HIs.

Phosphorus is important in the storage and transfer of energy obtained from photosynthesis and the metabolism of carbohydrates (McLaren & Cameron 1996). In wheat, P deficiency reduces plant leaf area by producing less and smaller leaves (Rodriguez et al., 1998), while the duration of leaf expansion is hardly affected. Low P also reduces number of fertile tillers, leaves and shoot dry weight (Kirschbaum and Tompkins, 1990; Bencze et al. 2000. On the other hand, K indirectly controls photosynthesis through regulation of stomata opening (Humble and Raschke, 1971; Asif et al. 2018), thus CO_2 assimilation. Furthermore, the regulation of stomata, concurrently, also controls transpiration rates. In this study, the

effects of eCO_2, P deficiency or K deficiency or their interactions on CHI, NHI and NuHI for spring wheat will be determined.

The aim of this chapter is to establish CHI, NHI and NuHIs for spring-sown wheat, cv. 'Discovery' grown under different CO_2, P and K fertiliser levels in a controlled environment. The objective is to determine the effects of CO_2 level (aCO_2; eCO_2), P and K fertiliser supply and their interactions on CHI, NHI, NuHIs and nutrient uptake patterns. The null hypothesis is that, neither CO_2 level nor P and K fertiliser supply will affect the CHI, NHI, NuHIs and nutrient uptake patterns. To do this, two experiments (Experiments 3 and 4) with the same treatments were undertaken in controlled environment, in 2019—20 season.

6.2 Material and methods

Experiments 3 and 4 were conducted across two Conviron BDW 120 plant growth rooms (Thermo Fisher Scientific, Auckland, New Zealand) at the Biotron facility (Beechey-Gradwell et al. 2018; Andrews et al. 2019; Beechey-Gradwell et al. 2020), Lincoln University, New Zealand. One growth room had a top-down airflow pattern, with controlled flow of filtered outdoor air, maintained at ambient CO_2 conditions (aCO_2; ~380 ppm CO_2). The second growth room had a similar airflow system but was maintained at elevated CO_2 (eCO_2; ~760 ppm) with G214 food grade CO_2 (BOC, Auckland, New Zealand) added as required. The two cabinets were previously tested for uniformity (Andrews et al. 2019). The CO_2 levels in the cabinets were measured continuously using PP Systems WMA-4 Gas Analysers (John Morris Scientific, Auckland, New Zealand). For both rooms, the lighting system consisted of 40 × 400 W metal halide bulbs (Venture Ltd, Mount Maunganui, New Zealand) in combination with 40 soft tone, soft white 100 W incandescent bulbs (Philips, Auckland, New Zealand) mounted behind a Perspex barrier 2.4 m above floor level. The photoperiod was 16 h with a PAR at the pot surface of ~800 µmol photons/m^2/s^1, confirmed with Sunfleck ceptometer (Model SF-80; Decagon Devices, WA 99163, USA) in both chambers. Light levels were ramped for 60 min to simulate dawn/dusk. Daytime relative humidity was maintained at 65% and night time humidity peaked at 80%. Temperature in both chambers were maintained under a 20/15°C, day/night regime, from sowing to final harvest.

Experiment 3 was sown on 11 September 2019 and Experiment 4 on 18 September 2019. Crops were harvested at anthesis and harvest maturity.

6.2.1 Experimental design and treatments

Experiments 3 and 4 were randomised complete block designs, consisting of 8 treatments (2 carbon dioxide (CO_2) levels, 2 P levels and 2 K levels) each and replicated four times. Spring wheat cultivar 'Discovery' was grown, at an N rate of 6 mM. The choice of genotype was based on results from Experiments 1 and 2 (Chapters 3—5) and N fertiliser rate from Andrews et al, (2019). In Chapters 3 and 5, 'Discovery' produced the highest AGB and grain yield at low and optimum N fertiliser supply. Furthermore, 'Discovery' is the only genotype out of the six used in this series of experiments (Chapter 3—5) with experimental results reported previously for K (Dawson et al. 2018) and N (Michel et al. 2018) fertiliser rates. The standard base nutrient solution has been described before, with the following composition (mol/m^3) (Cakmak et al. 1994): 0.88 K_2SO_4; 2.0 $Ca(NO_3)_2$; 0.25 KH_2PO_4; 1.0 $MgSO_4$; $1/10^2$ KC1; $1/10^2$ H_3BO_3; $2/10^2$ FeEDTA; $1/10^3$ $MnSO_4$; $1/10^3$ ZnSO4; $1/10^4$ $CuSO_4$; $1/10^3$ $(NH_4)_6$ MoO_{24}. The only change from this standard nutrient solution was the N rate, where 3.0 $Ca(NO_3)_2$ (6 mM) instead of 2.0 $Ca(NO_3)_2$ (4 mM) were used, based on the optimum for the previously reported wheat results from the same growth facility (Andrews et al. 2019). For P fertiliser treatments, P was supplied as NaH_2PO_4 at $1/10^2$ mol/m^3 instead of KH_2PO_4 to maintain the same K concentration with K_2SO_4 being supplied. These translated to 10 mmol/m^3 for the P deficient treatment and 250 mmol/m^3 for the P satiated crops. In K fertiliser treatments, K was added to nutrient solution at $5/10^2$ mol/m^3 K as K_2SO_4, and P and Cl were supplied as NaH_2PO_4 and NaCl, respectively. These translated to 50 mmol/m^3 for the K deficient treatment and 2010 mmol/m^3 for the K satiated crops. Combinations of these rates of application gave four fertiliser treatments: (1) Control, with optimum P (250 mmol/m^3) and K (2010 mmol/m^3) amounts, (2) K deficient, with K applied at 50 mmol/m^3, (3) P deficient, with P applied at 10 mmol/m^3, and (4) combined P & K deficient treatment.

The crop was grown in 'Dalton washed sand', in 40 cm long PVC tubes, with an inner diameter of 15.0 cm (total surface area = 176.8 cm^2). The bottoms are covered with perforated 2 L pots, pushed in an upright position, inside the PVC, to allow for free drainage. The PVC tubes were sat on solid 4 L pail containers, to capture all the mineral solutions draining through the bottom. The tubes were filled with 7.0 kg of washed sand. Before the experiment, the potting mix was analysed for fertility, and results showed: pH 7.3, and ≤1 mg/L for all the other nutrients [nitrate-N, ammonium-N, Olsen P, sulphate-S (S), K, Mg, Ca

and Na]. The crops were watered on alternate days with 300 ml of basal nutrient solutions (Cakmak et al. 1994) containing appropriate P and K treatments. The mineral solution captured by the 4 L pail containers was removed every week and thrown away.

6.2.2 Measurements

Crop development was monitored throughout the growing cycle. The wheat growth stage (GS) was monitored using the Zadoks scale (Zadoks et al. 1974). Biomass yield was determined at anthesis and harvest maturity. Plants were partitioned into leaf lamina and stem+sheath+ear at anthesis, and grain and straw (leaf + stem + chaff) at harvest maturity. Samples were dried in a forced air oven at 60°C until constant weight, generally ~72 hours and weighed. All samples were ground with a Cyclone Sample Mill (Udy Corporation, Fort Collins, Colorado, USA) to pass through a 1 mm screen and further processed for N amount (Section 3.5) and mineral nutrients (Section 4.2.1.2). Total nutrient amounts (mg/tube) were calculated as the product of DM yield (g/pot) and the nutrient concentration (mg/g) in the harvested crop (Muchow 1988).

The flag leaf area was estimated from measurements of its length (LL) and width (LW), twice after anthesis, as LL*LW*K, where K is a form factor (Bryson et al. 1997). In this study a K value of 0.83 (*Loc. cit*) was used, which was consistent with the 0.82—0.85 reported for old wheat genotypes (Owen 1968). Plant height was measured from the growing media (soil) surface to the top of the canopy (e.g. tip of the spike) using a tap measure.

6.2.3 Statistical analyses and calculations

Responses were analysed using a mixed model approach, fitted with REML as implemented in Genstat 18th edition. Assumptions were checked via standard residual plots and logarithmic transformation applied where needed. Fixed effects in the model were CO_2 level, P and K fertiliser supply and all interactions. Random effects accounted for the position (growthchamber.row*growthchamber.column) within the chambers. Carbon-dioxide level was aliased with growth chamber. Each variable was analysed separately. All effects discussed have a probability of $P \leq 0.05$. Measured variables are presented as separate experiments in tables and figures. However, in the text they are reported as averages when there were no significant differences between Experiments 3 and 4. In cases where there were difference between the experiments, the variable are reported

separately. Unless otherwise stated, interaction are given in the text when they are significantly different ($P \leq 0.05$).

6.3 Results

6.3.1 Crop harvest index (CHI)

The CHI did not differ ($P = 0.57$) between the two experiments, and was unaffected ($P = 0.11$) by CO_2 level at 0.44±0.01. However, CHI differed among the fertiliser treatments, higher for the control and K deficient crops at 0.51±0.01, compared with 0.37 when P was deficient. The CHI reported here for the control crops is consistent with the 0.55±0.01 reported in Experiment 1, but higher than the 0.42±0.03 for Experiment 2 (Section 6.1).

The shoot biomass yield at anthesis, total biomass at harvest maturity and grain yield were affected ($P < 0.001$) by the interactions between CO_2 level, P deficiency and K deficiency (Table 6.1). This was because they did not differ between the CO_2 levels for the P deficient crops, irrespective of the K levels. For example, shoot biomass at anthesis for P deficient crops was 3.92±0.95 g/tube for both CO_2 levels, while grain yield was 2.30±0.91 g/tube at both CO_2 levels. In contrast, shoot biomass yield at anthesis, total biomass at harvest maturity and grain yield increased ($P < 0.001$) with eCO_2 for the control and K deficient crops. For example, at anthesis, the shoot biomass yield for the control crops in Experiment 3 increased by 29.0% from 35.3±0.95 g/tube for the aCO_2 crops to 45.4 g/tube at eCO_2. Furthermore, biomass yield for the K deficient crops increased 45.0%, from 27.4 g/tube for the aCO_2 to 39.7 g/tube for the eCO_2. In Experiment 4, shoot biomass yield for the control crops increased by 25.0% from 30.9±0.95 g/tube for the aCO_2 to 38.6 g/tube for the eCO_2 and by 36.9% from 26.3 g/tube for the aCO_2 to 36.0 g/tube for the eCO_2 in the K deficient treatments.

Shoot biomass at anthesis differed among the fertiliser treatments, decreasing by ~87.4%, from 35.3±0.95 g/tube for the control to 4.45 for the P deficient crops for the aCO_2 crops in Experiment 3 (Table 6.1). Shoot biomass for the K deficient crops were intermediate, and decreased by 22.4% to 27.4±0.95 g/tube in Experiment 3. The crops grown at eCO_2 followed the same trend, decreasing ~90.6%, from an average of 42.0±0.95 g/tube for the control crops to 3.93 for the P deficient crops. Similarly, the K deficient crops decreased by 7.80% to

37.9±0.95 g/tube compared with the control. Similar trends were also observed in Experiment 4 (Table 6.1).

Table 6.1: Shoot biomass at anthesis, straw and grain yield and harvest maturity and crop harvest index (CHI) for wheat (cv. 'Discovery') grown with deficient phosphorus (P) and potassium (K) fertiliser at ambient and elevated carbon-dioxide in growth chambers, at Lincoln, New Zealand in 2019-20 growing season.

			Biomass and grain yield (g/tube)[1]			
	Treatments		Anthesis	Harvest maturity		
Exp	CO_2 level	Fertiliser rate	Shoot	Straw	Grain	CHI
3	Ambient	Control	35.3_a	27.9_a	31.0_a	0.53_a
		K deficient	27.4_b	25.6_b	28.4_b	0.53_a
		P deficient	4.45_c	3.69_c	2.37_c	0.38_b
		K*P deficient	3.75_c	3.24_c	1.83_c	0.35_b
	Mean		17.7_B	15.1_B	15.9_B	0.45_A
	Elevated	Control	45.4_a	37.4_a	35.2_a	0.49_a
		K deficient	39.7_b	30.7_b	31.3_b	0.51_a
		P deficient	3.75_c	4.11_c	2.57_c	0.38_b
		K*P deficient	3.75_c	5.06_c	2.89_c	0.37_b
	Mean		23.1_A	19.3_A	18.0_A	0.44_A
4	Ambient	Control	30.9_a	27.2_a	28.8_a	0.52_b
		K deficient	26.3_b	23.6_b	27.0_b	0.53_b
		P deficient	3.46_c	2.89_c	1.62_c	0.36_b
		K*P deficient	4.37_c	3.40_c	2.01_c	0.37_b
	Mean		16.3_B	14.3_B	14.9_A	0.44_A
	Elevated	Control	38.6_a	31.4_a	30.6_a	0.50_a
		K deficient	36.0_b	29.8_a	29.4_a	0.50_a
		P deficient	4.10_c	3.66_b	2.05_b	0.36_b
		K*P deficient	3.75_c	5.41_b	3.17_b	0.38_b
	Mean		20.6_A	17.6_A	16.3_A	0.43_A
	Significance: $LSD_{5\%}$ (P value[2])					
	Experiment (Exp)		0.96^{***}	1.13^{*}	0.62^{***}	0.03^{ns}
	CO_2 level (CO_2)		0.96^{***}	1.13^{***}	0.62^{***}	0.01^{ns}
	Fertiliser rate		1.35^{***}	1.60^{***}	0.88^{***}	0.02^{***}
	Exp* CO_2		1.35^{***}	1.60^{ns}	0.88^{***}	0.02^{ns}
	Exp*Fert.rate		1.91^{***}	2.26^{ns}	1.25^{**}	0.03^{ns}
	CO_2*Fert rate		1.91^{***}	2.26^{***}	1.25^{ns}	0.03^{ns}
	Exp* CO_2*Fert rate		2.71^{ns}	3.20^{***}	1.77^{ns}	0.04^{ns}

[1]Means with letter subscripts are compared within each column, and those with the same letters not different at $\alpha = 0.05$.

[2] * $P<0.05$, ** $P<0.01$, *** $P<0.001$ and ns = not significant

At harvest maturity, total biomass yield for the control crops in Experiment 3 increased from 58.9±0.91 g/tube for the aCO_2 crops to 72.6 g/tube under eCO_2 conditions (Table 6.1). In

Experiment 4, the total biomass for the control crops increased from 56.0±0.91 for the aCO_2 crops to 62.0 g/tube with eCO_2. This translated to 23.0% and 11.0% increase in Experiment 3 and 4, respectively. Furthermore, biomass yield for the K deficient crops increased by 15.7% from 52.3±2.58 g/tube for the aCO_2 to 60.5 g/tube for the eCO_2 crops for the two experiments. Total AGB also differed among the fertiliser treatments (Table 6.1), decreasing ~90.8%, from 57.4±1.28 g/tube for the control crops to 5.29 for the P deficient crops for both CO_2 levels. The AGB decreased by ~8.25%, with K deficiency (Table 6.1) from an average 57.0 g/tube for the control crops to 52.3 g/tube for the K deficient crops at aCO_2 and ~10.0%, from ~67.3 g/tube for the control crops to 60.6 g/tube for the K deficient crops at eCO_2

Grain yield for the control crops in Experiment 3 increased from 31.0±0.78 g/tube for the aCO_2 crops to 35.2 g/tube under eCO_2 conditions (Table 6.1), while in Experiment 4 the grain yield for the control crops increased from 28.8±0.91 to 30.6 g/tube with eCO_2. This translated to 14.0% and 6.0% increase in grain yield in Experiments 3 and 4, respectively.

Grain yield differed between CO_2 levels for the K deficient treatments, from an average of 27.7±0.87 g/tube for the aCO_2 to 30.4 g/tube for the eCO_2 crops across the two experiments. Grain yield also differed among the fertiliser treatments, and was lowest for the P deficient crops at 2.42 g/tube in Experiment 3 and 2.21 g/tube in Experiment 4. The K deficient crops were intermediate, in both experiments. The grain yield decreased by ~7.60% from 31.4±0.4 g/tube for the control crops to 29.0 for K deficient crops.

Biomass for the control crops at anthesis was on average 65.2±5.0% of the total biomass at harvest maturity (Table 6.1) and was affected ($P = 0.002$) by the fertiliser treatments, higher for the P deficient crops at 72.0% compared with 58.5% for the control and K deficient crops.

6.3.2 Yield components

6.3.2.1 Numbers of grains per unit area

The number of grains per tube (grain density) was unaffected by the CO_2 level, at an average of 422±13 grains per tube for the two experiments. However, grain density differed ($P \leq 0.001$) between experiments, higher in Experiment 3 at 444±13 compared with 400 grains per tube in Experiment 4. Grain density also differed among the fertiliser treatments, higher

for the control and K deficient crops. For example, in Experiment 3, grain density decreased by 88.4% for the P deficient crops and 7.60% for the K deficient crops, compared with the average of 825±24 grains/tube for the control crops. In Experiment 4, grain density for the control was 724±28, compared with 696 and 92.0 gains/tube for the K and P deficient crops, respectively.

Table 6.2: Number of grains (grain density; GD), thousand grain weight (TGW, g), number of fertile tillers, plant height (Ht; cm) and flag leaf size (FLS; cm^2) for wheat (cv. 'Discovery') grown with deficient phosphorus (P) and potassium (K) fertiliser at ambient and elevated carbon-dioxide in growth chambers, at Lincoln, New Zealand in 2019-20 growing season.

			Yield components[1]				
	Treatments						
Exp	CO_2 level	Fertiliser rate	GD	TGW	Tillers	Ht	FLS
3	Ambient	Control	834_a	37.2_a	17.3_a	71.6_a	17.5_a
		K deficient	789_a	36.0_a	14.5_b	72.3_a	16.4_a
		P deficient	107_b	21.9_b	5.25_c	44.8_b	8.18_b
		K*P deficient	80.3_b	22.9_b	5.50_c	37.0_b	6.12_b
	Mean		*453_A*	*29.5_B*	*11.0_A*	*56.4_B*	*12.1_B*
	Elevated	Control	815_a	43.2_a	16.8_a	88.8_a	20.3_a
		K deficient	735_a	42.6_a	15.3_a	86.3_a	19.9_a
		P deficient	90.5_b	28.5_b	5.25_b	52.5_b	9.95_b
		K*P deficient	103_b	28.6_b	6.0_b	50.0_b	9.64_b
	Mean		*436_A*	*35.7_A*	*11.0_A*	*70.7_A*	*15.3_A*
4	Ambient	Control	749_a	38.6_a	16.3_a	68.3_a	13.5_a
		K deficient	719_a	37.7_a	14.8_a	71.1_a	14.8_a
		P deficient	93.3_b	21.5_b	5.01_b	42.3_b	6.56_b
		K*P deficient	79.5_b	20.4_b	5.02_b	42.8_b	6.28_b
	Mean		*410_A*	*29.5_B*	*10.0_A*	*56.1_B*	*10.3_B*
	Elevated	Control	699_a	44.2_a	16.0_a	84.8_a	17.0_a
		K deficient	674_a	44.2_a	14.8_a	83.4_a	18.9_a
		P deficient	118_b	27.1_b	5.25_b	54.3_b	8.37_b
		K*P deficient	74.3_b	27.5_b	6.25_b	56.7_b	10.1_b
	Mean		*391_A*	*35.8_A*	*11.0_A*	*69.8_A*	*13.6_A*
	Significance: $LSD_{5\%}$ (P value[2])						
	Experiment (Exp)		0.96^{**}	1.13^{ns}	0.51^{ns}	1.84^{ns}	1.10^{**}
	CO_2 level (CO_2)		0.96^{ns}	1.13^{***}	0.51^{ns}	1.84^{***}	1.10^{***}
	Fertiliser rate		1.35^{***}	1.60^{***}	0.72^{***}	2.60^{***}	1.56^{***}
	Exp* CO_2		1.35^{ns}	1.60^{ns}	0.72^{ns}	2.60^{ns}	1.56^{ns}
	Exp*Fert.rate		1.91^{ns}	2.26^{ns}	1.02^{ns}	3.68^{ns}	2.20^{ns}
	CO_2*Fert rate		1.91^{ns}	2.26^{ns}	1.02^{ns}	3.68^{ns}	2.20^{ns}
	Exp* CO_2*Fert rate		2.71^{ns}	3.20^{ns}	1.44^{ns}	5.20^{ns}	3.11^{ns}

[1]Means with letter subscripts are compared within each column, and those with the same letters not different at $\alpha = 0.05$.

[2] * $P<0.05$, ** $P<0.01$, *** $P<0.001$ and ns = not significant

6.3.2.2 Thousand grain weight (TGW, g)

The TGW increased 21.0% with increasing CO_2 level in both experiments, from an average of 29.6±0.65 under aCO_2 conditions to 35.7 for the eCO_2 conditions. The TGW was also affected ($P \leq 0.001$) by fertiliser treatments, higher for the control and K deficient crops at an average of 40.5±0.93, compared with 25.0 for the P deficient crops, a decrease of 38.3%.

6.3.2.3 Number of fertile tillers and plant height

The number of fertile tillers and plant height did not differ ($P \geq 0.22$) between Experiments 3 and 4 at an average of 11.0±0.51 tillers and 62.9±1.83 cm (Table 6.2). The number of fertile tillers was unaffected ($P \geq 0.42$) by CO_2 level. However, the number of fertile tillers differed among the fertiliser treatments, highest for the control crops at 17.0±0.36 fertile tillers per tube, and lowest for the P deficient crop at 5.20 fertile tiller per tube. The K deficient crops were intermediate at 14.0 fertile tillers per tube. These represented a decrease of 65% for the P deficient crops and 18%, for the K deficient crops.

Plant height increased by 24% with increasing CO_2 level, from an average of 56.3±0.91 cm for the aCO_2 conditions to 69.6 cm under eCO_2 conditions (Table 6.2). Plant height also differed among the fertiliser rates, higher for the control and K deficient crops at an average of 78.4±1.29 cm, compared with 46.6 cm for the P deficient crops, a decrease of 41.0%.

6.3.2.4 Flag leaf area

Flag leaf area increased ($P < 0.01$) by 26.4% with increasing CO_2 supply, from 12.1±1.0 cm^2 for the aCO_2 to 15.3 cm^2 under eCO_2 conditions in Experiment 3 (Table 6.2), while in Experiment 4, it increased 32% from 10.3±0.53 cm^2 for the aCO_2 and to 13.6 cm^2 for the eCO_2 conditions.

Flag leaf area also differed ($P < 0.001$) among the fertiliser treatments (Table 6.2), being higher for the control and K deficient crops at 18.6±1.97 cm^2 compared with 7.63±1.97 for the P deficient crops in Experiment 3. In Experiment 4, flag leaf area decreased from 16.2±1.1 cm^2 for the control and K deficient crops to 8.64±1.1 cm^2 for the P deficient crop.

6.3.3 Accumulation and partitioning of nutrients

6.3.3.1 Nitrogen harvest indices (NHI)

The NHI was unaffected (P = 0.61) by CO_2 level at an average of 0.67±0.02 (Figure 6.1). However, NHI differed (P < 0.001) among the fertiliser treatments, higher for the control and K deficient treatments at 0.82±0.03 compared with 0.52 for the P deficient crops, irrespective of K rate. The NHI for the control crops were consistent with the 0.82±0.05 reported for Experiment 1 and 0.75±0.02 for Experiment 2.

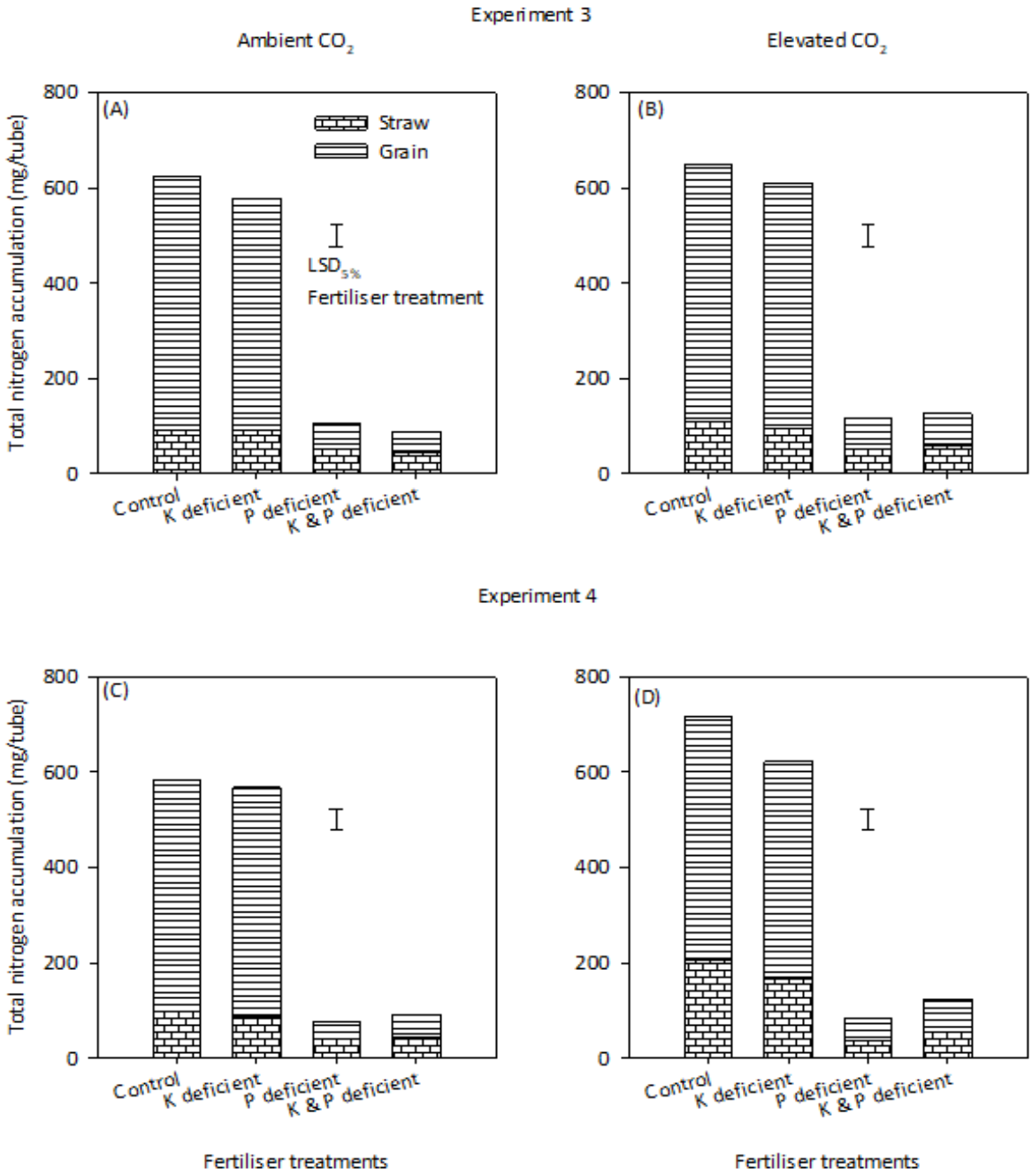

Figure 6.1: Total nitrogen accumulation (mg) for wheat (cv. Discovery) grown with deficient phosphorus (P) and potassium (K) fertiliser at ambient and elevated carbon dioxide in growth chambers, at Lincoln, New Zealand in 2019-20 growing season.

Total N accumulation at harvest maturity increased (P = 0.01) by 12% with increasing CO_2 supply, from 340±15.5 mg/tube for the aCO_2 to 381 mg/tube for the eCO_2 (Figure 6.1). Total herbage N accumulation also differed (P < 0.001) among the fertiliser treatments, highest for the control at 644±21.9 mg/tube, compared with 595 mg for the K deficient crops and 102 mg for the P deficient crops.

Grain N (N_g) accumulation increased (P < 0.05) by 5% with CO_2 supply, from 271±6.6 mg/tube for the aCO_2 to 284 mg at the eCO_2 (Figure 6.1). The N_g accumulation also differed (P < 0.001) among the fertiliser treatments, highest for the control crops at 518±9.4 mg/tube, compared with 485 mg for the K deficient crops and 54 mg for the P deficient crops.

6.3.3.2 Phosphorus harvests indices (PHI)

The PHI was unaffected by CO_2 level at an average of 0.77±0.01 (Figure 6.2). However, PHI was higher (P < 0.001) for the control and K deficient crop at 0.87±0.03 in Experiment 3 and 4, and lower for the P deficient crops at 0.69±0.03 in Experiment 3 and 0.62 in Experiment 4. The high PHI for the control crops was consistent with the value of 0.86±0.01 reported in Experiment 1 but higher than the 0.67±0.02 for Experiment 2.

At harvest maturity, total herbage P accumulation was unaffected (P = 0.12) by CO_2 level, at an average of 20.3±1.1 mg/tube (Figure 6.2). However, total P accumulation was higher in Experiment 3 at 21.5±1.1 mg/tube compared 19.1 mg for Experiment 4. Furthermore, total P accumulation differed (P < 0.001) among the fertiliser treatment. In Experiment 3, total P was higher for the control crops at 46.1±1.6 mg/tube compared with 35.6 mg and 2.14 mg for the K and P deficient crops, respectively. In Experiment 4, total herbage P was 43.1±1.4 mg/tube for the control crops compared with 30.1 mg and 1.68 mg for the K and P deficient crops, respectively.

Grain P accumulation was unaffected (P = 0.19) by CO_2 level, at an average of 17.5±0.88 mg/tube (Figure 6.2). However, grain P accumulation differed (P < 0.001) among the fertiliser treatments, higher for the control crops at 41.0±1.3 mg/tube compared with 31.4 mg for the K deficient crops and 1.45 mg for the P deficient crops in Experiment 3. In Experiment 4, grain P accumulation was also higher for the control crops at 37.0±1.3

mg/tube compared with 25.8/tube mg for the K deficient crops and 1.30 mg/tube for the P deficient crops.

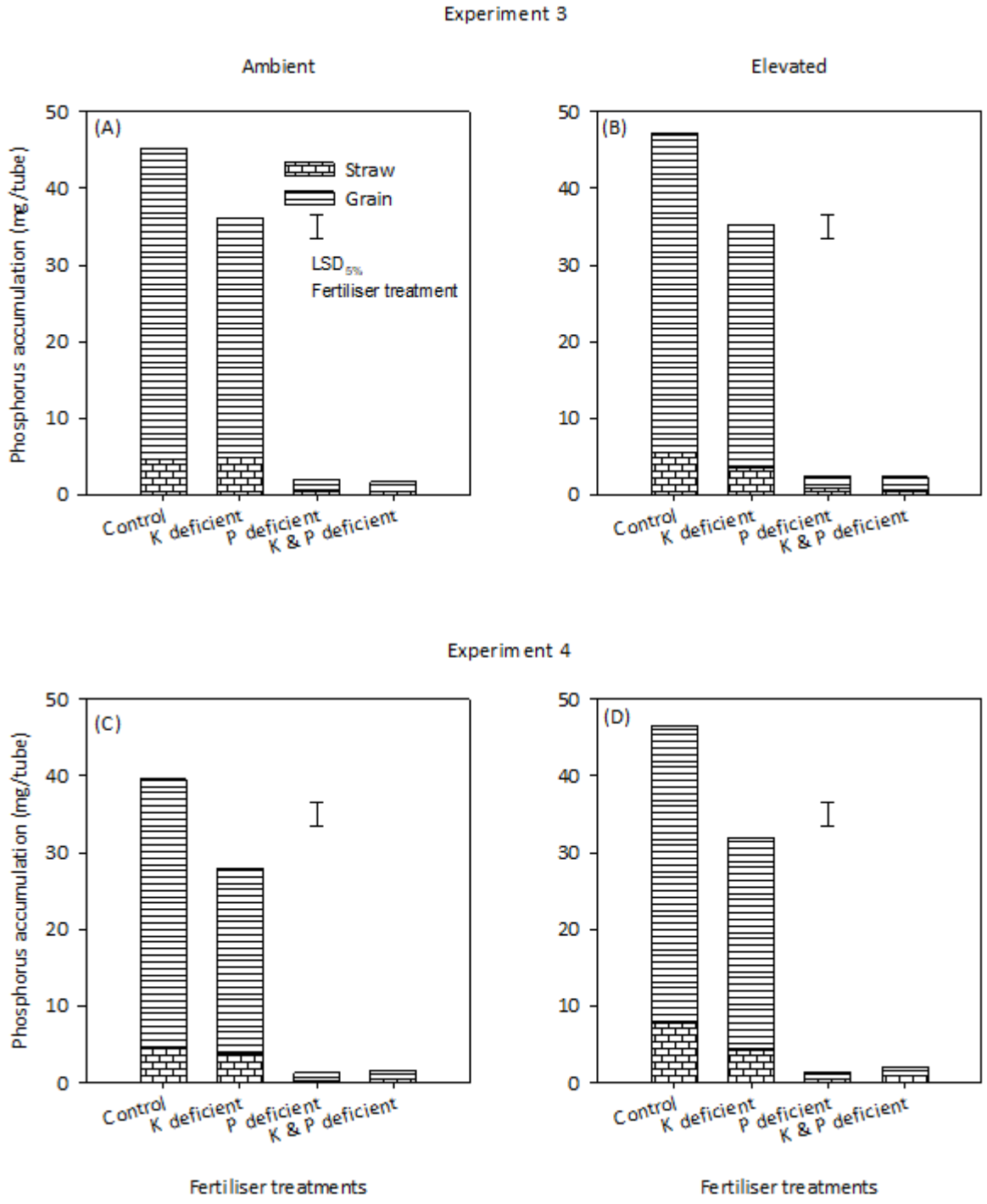

Figure 6.2: Phosphorus accumulation (mg/tube) at anthesis (A—B), and at harvest maturity (C—F) and phosphorus harvest index (PHI) (C—F) for wheat (cv. Discovery) grown with deficient phosphorus (P) and potassium (K) fertiliser at ambient and elevated carbon-dioxide in growth chambers, at Lincoln, New Zealand in 2019-20 growing season.

6.3.3.3 Potassium harvests indices (KHI)

The KHI decreased ($P < 0.05$) by 18% with increasing eCO_2, from an average of 0.22±0.03 at aCO_2 to 0.18 for the eCO_2 crops in both experiments (Figure 6.3). Furthermore, KHI differed ($P < 0.001$) among the fertiliser treatments, higher for the control and K deficient crops at 0.24±0.023 compared with 0.14 for the P deficient crops.

At harvest maturity, the total herbage K accumulation did not differ between the two experiments (Figure 6.3), at 586±10.6 mg/tube for the control crops. Total herbage K accumulation increased ($P < 0.001$) by 13% with increasing CO_2 level, from 551±21.3 mg/tube for the aCO_2 to 621 mg at eCO_2 conditions. Similarly, total K accumulation for the K deficient crops increased by 7%, from 343±21.3 mg/tube for the aCO_2 to 367 mg/tube for the eCO_2. Potassium accumulation also differed among the fertiliser treatments, highest for the control crops at 586±15.1 mg/tube, compared with 355 mg/tube for the K deficient crops and 58.5 mg/tube for the P deficient crops. This translated to a decrease of 39.4% for the K deficient crops and 90% for P deficient crops, compared with the control crops.

The grain K accumulation differed ($P < 0.001$) between the experiments (Figure 6.3). Grain K accumulation also differed among the fertiliser treatments, being higher ($P < 0.001$) for the control crops at 139±2.9 mg/tube compared with 111 mg/tube for the K deficient crops and 8.30 mg/tube for the P deficient crop in Experiment 3. In Experiment 4, K accumulation was 121±2.9 mg/tube for the control crops, compared with 97.4 mg/tube for the K deficient crops and 6.60 mg/tube for the P deficient crops.

6.3.3.4 Nutrient harvest indices (NuHIs) for other nutrients

All other NuHIs, were unaffected ($P \geq 0.12$) by CO_2 level, except for CuHI which decreased 39.1%, from 0.23±0.03 for the aCO_2 to 0.14 for the eCO_2. All NuHIs were higher for the control and K deficient crops, compared with the P deficient crop, except for CaHI and CuHI (Table 6.3), which were unaffected by fertiliser treatment. Macro-nutrient HIs decreased by 22—34%, while micro-nutrients HIs were more variable at 28—65%. For example, SHI for the control and K deficient crops was 0.45±0.03 compared with 0.35 for the P deficient crops, while ZnHI was also high for the control and K deficient crops at 0.51±0.01, compared with 0.37 for the P deficient crops.

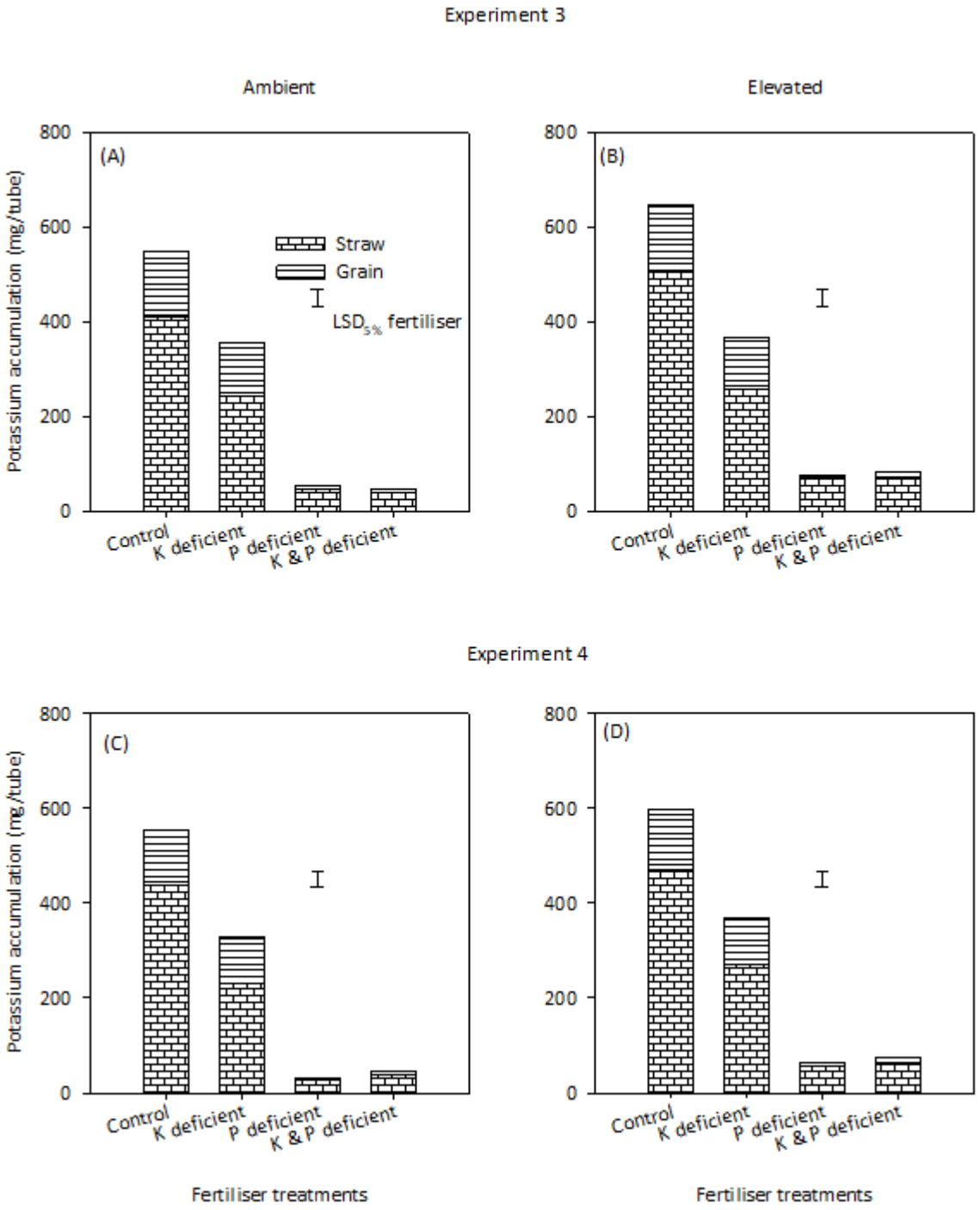

Figure 6.3: Potassium accumulation (mg/tube) at anthesis (A—B), and at harvest maturity (C—F) and potassium harvest index (KHI) (C—F) for wheat (cv. Discovery) grown with deficient phosphorus (P) and potassium (K) fertiliser at ambient and elevated carbon-dioxide in growth chambers, at Lincoln, New Zealand in 2019-20 growing season.

Table 6.3: Nutrient harvest index (NuHI) for wheat (cv. Discovery) grown with deficient phosphorus (P) and potassium (K) fertiliser at ambient and elevated carbon-dioxide ingrowth chambers, at Lincoln, New Zealand in 2019-20 growing season.

Exp	Treatments		Nutrient harvest index (NuHI)[1]						
	CO_2 level	Fert. rate	SHI	CaHI	MgHI	FeHI	MnHI	ZnHI	CuHI
3	Ambient	Control	0.45_a	0.21_a	0.52_a	0.53_a	0.53_a	0.53_a	0.27_a
		K deficient	0.47_a	0.18_a	0.45_a	0.53_a	0.47_a	0.53_a	0.25_a
		P deficient	0.34_b	0.08_b	0.22_b	0.38_b	0.22_b	0.38_b	0.27_b
		K*P deficient	0.31_b	0.06_b	0.19_b	0.35_b	0.20_b	0.35_b	0.23_b
	Mean		*0.39*$_A$	*0.13*$_A$	*0.35*$_A$	*0.45*$_A$	*0.35*$_A$	*0.45*$_A$	*0.25*$_A$
	Elevated	Control	0.46_a	0.13_a	0.40_a	0.88_a	0.43_a	0.49_a	0.14_a
		K deficient	0.39_a	0.12_a	0.36_a	0.71_a	0.38_a	0.51_a	0.13_a
		P deficient	0.36_b	0.12_b	0.30_b	0.08_b	0.22_b	0.38_b	0.22_b
		K*P deficient	0.36_b	0.17_b	0.37_b	0.26_b	0.22_b	0.37_b	0.18_b
	Mean		*0.39*$_A$	*0.13*$_A$	*0.36*$_A$	*0.48*$_A$	*0.31*$_A$	*0.44*$_A$	*0.17*$_B$
4	Ambient	Control	0.46_a	0.14_a	0.41_a	0.74_a	0.49_a	0.52_a	0.21_a
		K deficient	0.49_a	0.21_a	0.45_a	0.72_a	0.35_a	0.53_a	0.15_a
		P deficient	0.34_b	0.09_b	0.24_b	0.17_b	0.18_b	0.37_b	0.14_b
		K*P deficient	0.44_b	0.17_b	0.34_b	0.31_b	0.28_b	0.36_b	0.27_b
	Mean		*0.43*$_A$	*0.15*$_A$	*0.36*$_A$	*0.50*$_A$	*0.33*$_A$	*0.45*$_A$	*0.20*$_A$
	Elevated	Control	0.47_a	0.09_a	0.42_a	0.75_a	0.34_a	0.50_a	0.10_a
		K deficient	0.42_a	0.10_a	0.40_a	0.72_a	0.37_a	0.50_a	0.11_a
		P deficient	0.32_b	0.12_b	0.38_b	0.17_b	0.20_b	0.38_b	0.12_b
		K*P deficient	0.31_b	0.07_b	0.32_b	0.24_b	0.28_b	0.36_b	0.14_b
	Mean		*0.38*$_A$	*0.10*$_A$	*0.38*$_A$	*0.44*$_A$	*0.30*$_A$	*0.43*$_A$	*0.12*$_B$
Significance: $LSD_{5\%}$[2]									
Exp			0.04^{ns}	0.03^{ns}	0.05^{ns}	0.09^{ns}	0.05^{ns}	0.01^{ns}	0.06^{ns}
CO_2 level			0.04^{ns}	0.03^{ns}	0.05^{ns}	0.09^{ns}	0.05^{ns}	0.01^{ns}	0.06^{**}
Fert.			0.06^{***}	0.04^{ns}	0.06^{***}	0.13^{**}	0.07^{***}	0.02^{***}	0.08^{ns}
Exp* CO_2			0.06^{ns}	0.04^{ns}	0.06^{ns}	0.13^{ns}	0.07^{ns}	0.02^{ns}	0.08^{ns}
Exp*Fert.			0.08^{ns}	0.06^{ns}	0.09^{ns}	0.18^{ns}	0.10^{ns}	0.03^{ns}	0.11^{ns}
CO_2*Fert.			0.08^{ns}	0.06^{ns}	0.09^{ns}	0.18^{ns}	0.10^{ns}	0.03^{ns}	0.11^{ns}
Exp* CO_2*Fert			0.11^{ns}	0.08^{ns}	0.13^{ns}	0.25^{ns}	0.14^{ns}	0.04^{ns}	0.16^{ns}

[1]Means with letter subscripts are compared within each column, and those with the same letters not different at $\alpha = 0.05$.

[2] * $P<0.05$, ** $P<0.01$, *** $P<0.001$ and ns = not significant

6.3.4 Nutrient concentration

At anthesis, nutrient concentrations were within or above the reported threshold concentrations for optimal crop growth (Marschner 1995; Reuter et al. 1997), except for N, P and K (Table 6.4; Appendix 6.1). Grain N concentration for the control crops were within ranges of 1.96—2.04% (equivalent to GPC of 11.2—11.6%) (Appendix 6.2), and adequate for human health (Reuter & Robinson 1997; Liu et al. 2014) for all, except for Fe, Mn and Cu.

Table 6.4: Phosphorus (P) and potassium (K) concentration for shoot at anthesis, total shoot and grain for wheat (cv. Discovery) grown with deficient P and K fertiliser rate (Fert.) at ambient and elevated carbon-dioxide in growth chambers, at Lincoln, New Zealand in 2019-20 growing season.

			Phosphorus (P) and potassium (K) concentration (%)[1]					
Exp	Treatments		Anthesis		Harvest maturity		Grain	
	CO_2 level	Fert. rate	P%	K%	P%	K%	P%	K%
A	Ambient	Control	0.12_a	1.47_a	0.077_a	0.93_a	0.13_a	0.44_a
		K deficient	0.12_a	1.08_d	0.067_a	0.75_b	0.11_b	0.40_b
		P deficient	0.06_b	1.36_b	0.032_b	0.92_a	0.06_c	0.36_c
		K*P deficient	0.06_b	1.22_c	0.032_b	0.91_a	0.06_c	0.35_c
	Mean		*0.09_A*	*1.28_A*	*0.052_A*	*0.88_A*	*0.09_A*	*0.39_A*
	Elevated	Control	0.14_a	1.48_a	0.065_a	1.0_a	0.12_a	0.40_a
		K deficient	0.13_a	0.98_d	0.057_a	0.60_b	0.10_b	0.35_b
		P deficient	0.05_b	1.36_b	0.037_b	1.10_a	0.06_c	0.32_c
		K*P deficient	0.06_b	1.26_c	0.032_b	1.0_a	0.06_c	0.32_c
	Mean		*0.09_A*	*1.27_A*	*0.048_A*	*0.93_A*	*0.09_A*	*0.35_B*
B	Ambient	Control	0.12_a	1.48_a	0.071_a	1.0_a	0.12_a	0.40_a
		K deficient	0.10_b	1.12_d	0.055_b	0.65_c	0.10_a	0.36_b
		P deficient	0.06_c	1.39_b	0.028_c	0.81_b	0.06_b	0.32_c
		K*P deficient	0.06_c	1.24_c	0.032_c	0.84_b	0.06_b	0.33_c
	Mean		*0.09_A*	*1.31_A*	*0.047_A*	*0.82_A*	*0.08_A*	*0.35_A*
	Elevated	Control	0.11_a	1.53_a	0.076_a	1.0_a	0.13_a	0.41_a
		K deficient	0.10_a	0.87_d	0.054_b	0.63_c	0.10_b	0.33_b
		P deficient	0.06_b	1.35_b	0.025_c	1.0_a	0.04_c	0.28_c
		K*P deficient	0.06_b	1.27_c	0.024_a	0.82_b	0.04_c	0.27_c
	Mean		*0.08_A*	*1.25_B*	*0.045_A*	*0.85_A*	*0.08_A*	*0.32_B*
Significance: $LSD_{5\%}$[2]								
Exp			0.003^{***}	0.04^{ns}	0.004^{ns}	0.07^{ns}	0.01^{ns}	0.01^{***}
CO_2 level			0.003^{ns}	0.04^{ns}	0.004^{ns}	0.07^{ns}	0.01^{ns}	0.01^{***}
Fert.			0.005^{***}	0.05^{***}	0.01^{***}	0.10^{***}	0.01^{***}	0.02^{***}
Exp* CO_2			0.005^{ns}	0.05^{ns}	0.01^{ns}	0.10^{ns}	0.01^{ns}	0.02^{ns}
Exp*Fert.			0.006^{ns}	0.07^{ns}	0.01^{ns}	0.13^{ns}	0.01^{ns}	0.03^{ns}
CO_2*Fert.			0.006^{ns}	0.07^{ns}	0.01^{ns}	0.13^{ns}	0.01^{ns}	0.03^{ns}
Exp* CO_2*Fert			0.01^{ns}	0.10^{ns}	0.01^{ns}	0.19^{ns}	0.02^{ns}	0.0^{ns}

[1]Means with the letter subscripts are compared within each column, and those with the same letters not different at $\alpha = 0.05$.

[2] * P<0.05, ** P<0.01, *** P<0.001 and ns = not significant

6.3.4.1 Phosphorus concentration

Phosphorus concentration was unaffected (P = 0.44) by the CO_2 level at anthesis and harvest maturity (Table 6.4). However, P concentration differed (P < 0.001) among the fertiliser treatments. At anthesis, P concentration was high for the control and K deficient crops at an average of 0.12% compared with 0.06% for the P deficient crops for both experiments. At

harvest maturity, P concentration was ~50% higher for the control and K deficient crops compared with the 0.03% for the P deficient crops. Grain P concentration was unaffected ($P = 0.78$) by the CO_2 level (Table 6.4). However, grain P concentration was higher ($P < 0.001$) for the control at 0.12±0.01% compared with 0.09% for the K and 0.05% for the P deficient crops.

6.3.4.2 Potassium concentration

Potassium concentration was unaffected ($P = 0.15$) by the CO_2 level at anthesis and harvest maturity (Table 6.4). However, at anthesis, K concentration was higher ($P < 0.001$) for the control and P deficient crops at 1.50±0.03% compared with 0.86% for the K deficient crops. At harvest maturity, K concentration was lower ($P < 0.001$) at 0.06±0.01% for the K deficient crops, compared with an average of 1.0±0.01% the control and P deficient crops, in both experiments. Potassium concentration decreased ($P < 0.05$) with developmental stage (Table 6.4), from 1.28±0.03% at anthesis, to 0.86% at harvest maturity.

Grain K concentration decreased ($P < 0.001$) from 0.37±0.01% for the aCO_2 crop to 0.33% for the eCO_2 crops (Table 6.4). Grain K concentration was lowest ($P < 0.001$) for the K deficient crops at 0.31±0.01% compared with 0.41% for the control crops and 0.36% for the P deficient crops.

6.3.4.3 Crop concentrations for other nutrients

At anthesis and harvest maturity, herbage nutrient concentration (%; mg/kg DM) for other nutrients differed ($P \leq 0.02$) among the fertiliser treatments for all nutrients, except for N at harvest maturity (Table 6.4, Appendix 6.1). At anthesis, the concentrations of all nutrients were unaffected by eCO_2 level, except S and Fe. Sulphur concentration increased from 0.28±0.003% for the aCO_2 to 0.31% for the eCO_2 crops, while Fe concentration increased from 233±18.5 mg/kg for the aCO_2 to 266 mg/kg, for the eCO_2 crops. At harvest maturity, only N and Mg concentration were affected by CO_2 level. N decreased from 0.88±0.01% for the aCO_2 to 0.83 for the eCO_2 and Mg decreased from 0.16±0.01% for the aCO_2 to 0.14 for the eCO_2. Nutrient concentrations were higher for the P deficient crops in the herbage for all nutrient (Appendix 6.2), except for S at anthesis, and N and S at harvest maturity.

Grain nutrient concentration decreased ($P < 0.05$) with increasing CO_2 level for N, S, Ca and Mg, increased for Fe, but was unaffected ($P \geq 0.12$) by CO_2 for Mn, Zn and Cu (Table 6.4,

Appendix 6.2). For example, grain S concentration decreased from 0.75±0.01% for the aCO_2 to 0.70 at eCO_2. In contrast, Fe concentration increased from 48.4±3.11 mg/kg at aCO_2 to 67.7 mg/kg at eCO_2 level. In the grain: N, Fe, Zn, and Mn concentration were higher for the P deficient crops (Appendix 6.2). These relationships reflects the level of high nutrient uptake against total biomass, which resulted in the high concentration for the low biomass treatments.

6.3.5 Crop accumulation of other nutrients

At anthesis, there were interactions ($P < 0.001$) between CO_2 level, P deficiency and K deficiency for all nutrient elements (Appendix 6.3), except for Zn and Cu. This was because nutrient accumulation was unaffected by CO_2 level for the P deficient crops. In contrast, nutrient accumulation increased with CO_2 level for the control and K deficient crops. For example, S accumulation for the control crops increased by 57%, from 97.5±2.72 mg/tube for the aCO_2 to 153 mg/tube for the eCO_2 in Experiment 3, and by 23% from 91.1±2.72 for the aCO_2 to 112 mg/tube for the eCO_2 in Experiment 4. At harvest maturity, nutrient accumulation increased ($P < 0.05$) with CO_2 level for all nutrients, except for Mg and Zn (Appendix 6.3).

At anthesis and harvest maturity, nutrient accumulation also differed among the fertiliser treatments and were higher for the control crops compared with the P deficient crops (Appendix 6.3). Nutrient accumulation for the K deficient crops was intermediate, except for Ca and Mg, which were highest for the K deficient crops compared with the control and P deficient crops. At anthesis Ca accumulation was 58.4±1.18 mg/tube for the K deficient crops, compared with 54.9 mg/tube for the control crops and 11.4 for the P deficient crops in Experiment 3. At harvest maturity, Ca accumulation was 75.3±1.18 mg/tube for the K deficient crops compared with 61.0 mg/tube for the control crops and 13.2 mg/tube for the P deficient crops in Experiment 3. Similarly, Mg accumulation was 75.8±1.18 mg/tube for the K deficient crops, compared with 67.2 mg/tube for the control crops and 9.6 mg/tube for the P deficient crops in Experiment 4.

For the grain component all mineral elements were affected ($P < 0.05$) by fertiliser application, lower for the P deficient crops and higher for the control treatments. The K deficient crops were intermediate.

The proportion of total nutrient for the control crops accumulated at anthesis compared with the total at harvest maturity (Appendix 6.3), was on average 61.0±10% for N, 100±17% for P, 106±14% for K, 47.5±8.0% for S, 83.0±24% for Ca, 47.3±13% for Mg, 56.0±23% for Fe, 74.0±10% for Mn, 60±27% for Zn, and 38.0±20% for Cu. These figures are consistent with Experiment 1, except for P and Cu, both higher than Experiment 1.

6.4 Discussion

The objective of this study was to determine the effects of CO_2 level (aCO_2; eCO_2), P and K fertiliser supply on CHI, NHI and NuHIs for spring-sown wheat, cv. 'Discovery'. Results show that CHI and all NuHIs were unaffected by CO_2 level (except KHI and CuHI) or K deficiency. However, CHI and all NuHIs were severely impacted by P deficiency, with CHI decreasing 27.5%, from 0.51±0.01 for the control/ K deficient crops to 0.37 for the P deficient crops, while NHI decreased 36.5%, from 0.82±0.03 for the control/ K deficient crops to 0.52 for the P deficient crops. Total AGB and grain yield increased with eCO_2, and were also severely limited by P deficiency. However, AGB and grain yield were sparingly affected by K deficiency, decreasing by 10% and 7.6%, respectively, compared with the control crops. The proportion of nutrients for the control crops at anthesis compared with the total at harvest maturity were 47.0—60.0% for Mg, S, Fe, Zn and N; 74.0—100% for Mn, Ca, P and K and ~38.0% for Cu. These proportions were similar to Experiment 1 except for N and P, which were lower and higher, respectively, in Experiments 3 and 4. Similar proportions were also reported for the glasshouse, except for Mn and S, which were lower and higher, respectively, in Experiment 3 and 4.

The CHI reported for the control crops in the current study of 0.51±0.01 was consistent with the 0.54±0.01 reported for the same genotype in Experiment 1 (Figure 3.4) but higher than the 0.42±0.04 reported in Experiment 2 (Table 5.1). The low CHI for the glasshouse could be attributed to the high temperatures around anthesis (Sections 5.4.1; 6.1), which have been shown to reduce grain density (Wheeler et al. 1996; Ferris et al. 1998), resulting in lower grain yields. These CHI values are consistent with the 0.40—0.50 reported for spring wheat (Goudriaan & de Ruiter 1983; Dai et al. 2016).

6.4.1 Carbon-dioxide (CO_2) level

This study suggests that CHI does not respond to eCO_2, in the absence of water and/ or N stress, for the genotype examined here. Previous studies on effects of eCO_2 on CHI have been inconsistent, unaffected (e.g. Broberg et al. 2019), declined when associated with N stress (Sionit et al. 1981; Goudriaan & de Ruiter 1983) and increased when associated with water stress (Gifford 1979; Sionit et al. 1980). Both water and N were not limiting in the current study, hence the non-response of CHI to eCO_2.

The AGB yield for the control crops increased 11—23% with eCO_2 (Section 6.3.1), which was consistent with the 8—24% increase reported in previous studies for spring (Högy et al. 2009a; Högy et al. 2009b) and winter (Batts et al. 1998) wheat. Furthermore, grain yield increased by 6—14% with eCO_2 (Table 6.1), which was also consistent with the 7—10% increase reported previously (Amthor 2001; Högy et al. 2009b). The fact that eCO_2 affected yield and not CHI (Table 6.1) showed that yield was affected more by the changes in total production than the partitioning of photosynthate to the grain. The higher AGB yields at eCO_2, were reflected by increased plant height and a larger flag leaf area for the eCO_2 treatments (Table 6.2), while grain yield was explained by TGW, as grain density was unaffected by CO_2 level. Specifically, increased plant height under eCO_2, meant higher AGB and more resources for the development of the grain, hence the higher TGW. Furthermore, a larger flag leaf area under eCO_2, meant a greater leaf surface for intercepting IPAR and the resultant higher AGB and grain yields. Similar results have been reported for winter (Maphosa et al. 2019) and spring wheat (Li et al. 2004). Higher yields for taller plants (Table 6.2), were also reported in Experiment 1 (Table 3.6) and in previous studies (Addisu et al. 2010; Gooding et al. 2012; Casebow et al. 2016; Maphosa et al. 2019).

The NuHIs were affected by CO_2 level for KHI and CuHI only (Figures 6.1—6.3; Table 6.3), which decreased at eCO_2. These results are inconsistent with previous reports, which showed an increase in KHI and MgHI, and a decrease in SHI (Manderscheid et al. 1995) and an increase in NHI (Hazra et al. 2019). Furthermore, PHI was unaffected by eCO_2 (Manderscheid et al. 1995), similar to the current studies. A comprehensive literature search did not find any further reports on the effects of eCO_2 on NuHIs. The nutrient uptake for all nutrients was at least as great under eCO_2 in comparison with aCO_2 (Figure 6.2, 6.3; Table 6.3). Thus, reduced KHI and CuHI were due to the reduced nutrient translocation to the

grain. The magnitude of differences for KHI of ~0.22 for the aCO_2 and 0.18 for the eCO_2 and 0.23 to 0.14, respectively, for CuHI was consistent between experiments. For the other nutrients, the total uptake was greater for eCO_2 for N, S, Ca, Fe and Mn, but amounts were unchanged for P, Mg and Zn (Appendix 6.3). As NuHIs were similar for N, S, Ca, Mg, Fe, and Mn, then nutrient translocation to the grain was similar under aCO_2 and eCO_2. However, carbon translocation to the grain was greater under eCO_2 (Table 6.1), thus, the grain concentration for these elements decreased with eCO_2 (Appendix 6.2) as reported previously (Manderscheid et al. 1995). The decreasing nutrient concentrations could be attributed to the dilution effects due to the increased accumulation of carbohydrates, as crops grown under eCO_2 produced more grain yield (Table 6.1) (Taub & Wang 2008; McGrath & Lobell 2013).

6.4.2 Potassium fertiliser rate

The CHI, NHI and all NuHIs were unaffected by K deficiency in both experiments. The lack of response of CHI (Table 6.1), was inconsistent with recent reports for wheat grown in a controlled environment (Asif et al. 2018). These authors reported severe yield reduction with K deficiency, of ~50% reduction for the straw biomass and ~67% reduction for the grain yield. In the current study, AGB and grain yield were sparingly affected by K deficiency, decreased by 10% and 7.6%, respectively, compared with the control crops (Section 6.3.1). Therefore, the effects of K deficiency on CHI, NHI and NuHI may be dependent on its severity on yield.

The non-response to K deficiency for the measured attributes (CHI, NHI and NuHIs), could be a result of K substitution by sodium (Na), as reported previously (Wakeel et al. 2011). Sodium can partially replace K (Flowers et al. 1977), particularly in its non-specific metabolic functions (Wakeel et al. 2011), e.g. osmotic functions in the vacuole. Thus under K deficiency, addition of Na can promote plant growth. In the current experiment, in the K deficient treatment, P was applied as NaH_2PO_4 instead of KH_2PO_4 (Section 6.2.1). The applied Na could have replaced the non-specific functions of K and allowed crops to grow normally, as the measured attributes were all similar to the control crops, except for K concentration, which was lower under K deficient crops and the minor yield differences.

The higher Ca and Mg concentration for the K deficient crops (Appendix 6.1), and resultant higher accumulation values, have been attributed to ion antagonism (Mengel et al. 2001) as there is an established inverse relationship between concentration of K and that of other cations (Ragab 1979). This had been reported for oats and a range of vegetable seedlings (Freeman 1967). When K is deficient, plants will take up more of the divalent cations, hence the greater accumulation of Ca and Mg reported here, a result of reduced competition from K ions.

6.4.3 Phosphorus fertiliser rate

Results under P deficiency were markedly different from those under eCO_2 and K deficiency. Phosphorus deficiency severely decreased growth, CHI and NuHIs (except for CaHI and CuHI). However, temporal nutrient uptake was similar under P deficiency as for the other treatments

Phosphorus deficiency decreased AGB by ~90.0%, from 57.4±1.28 g/tube for the control crops to 5.29 for the P deficient. These effects of P deficiency on AGB have been reported in previous studies (Goudriaan & de Ruiter 1983; Manoj et al. 2012), but the severity of reduction was more intense in the current study compared with 45—58% in the previous studies (*Loc. cit*). The effects of P deficiency on grain yield were attributed to its severe effects on yield components (Table 6.2) as they decreased under P deficiency (Section 6.3.2). The combined low grain density and TGW under P deficiency, led to the extremely low grain yields. Furthermore, the reduced plant height and number of fertile tillers under P deficiency meant less AGB compared with the control crops (Table 6.2). The low number of fertile tillers and flag leaf area meant reduced canopy cover and hence reduced surface for intercepting IPAR, which resulted in low AGB and grain yield. This is the first report we can find on the effects of P deficiency on yield components, for wheat crop grown under controlled environments. In other environments, these yield components have been shown to be critical in yield determination, e.g. Maphosa et al. (2019), Addisu et al. (2010) and Gooding et al. (2012).

In addition to the severe reduction in growth, P deficiency decreased CHI by 27.5% from 0.51±0.01 for the control/ K deficient crops to 0.37 for the P deficient crops. These results are consistent with previous reports that showed a decrease of ~20% in a glasshouse

experiment (Goudriaan & de Ruiter 1983) and ~10% in phytotron growth chambers (Manoj et al. 2012).

Phosphorus deficiency decreased NuHIs, except for CaHI and CuHI. Nutrient uptake for all nutrients was severely reduced by P deficiency (Appendix 6.3). The nutrient uptake relative to the carbon fixed increased under P deficiency. This resulted in greater nutrient concentration of the AGB (Appendix 6.1). However, relative to carbon, less nutrients were translocated to the grains, resulting in reduced NuHIs (Table 6.3).

For the control crops, there was a near isometric uptake of most nutrients, with 47.0—60.0% of N, Mg, S, Fe and Zn taken up at anthesis. In contrast, ~38.0% of Cu and 74—100% of Mn, Ca, P and K were accumulated pre—anthesis. These results meant that the temporal uptake for Fe, Mg and Zn was consistent among the four experiments and related to the biomass accumulation of 50.0—65.0%. The lower Cu and higher K and Ca were also consistent across the four experiments (Sections 4.6.2, 5.4.3 and 6.3.7). The large proportion of Ca and K accumulated during early growth has been reported for wheat (Hocking 1994; Gaj & Rebarz 2014; Hamnér et al. 2017), grain sorghum (Hocking 1993) and maize (Karlen et al. 1988; Ciampitti et al. 2013; Ciampitti & Vyn 2013). The substantial uptake of most the nutrients after anthesis across the four experiments, except for Ca and K, has implications for nutrient management. Some of these nutrients are highly soluble (e.g. S and N) and can easily be leached in soils in arable lands (McLaren & Cameron 1996)) and therefore could benefit from split application during the growing season.

6.5 Conclusions

Overall, CO_2 level had little effects on CHI, NHI and NuHIs (except KHI and CuHI). For example, average CHI was 0.44±0.01. These relatively small changes were associated with an increase in growth. Potassium deficiency had no effect on CHI and NuHIs, and had little impact on growth but substantially reduced K concentration. Phosphorus deficiency substantially affected CHI, NuHIs and nutrient concentration. These effects were associated with P deficiency's severe effect on growth. These finding agree with the literature of growth reduction under P deficiency of ~50%. It is possible that substantial effects on CHI and NuHIs occurs only when growth is severely affected. Biomass yield increased 11—23% with eCO_2, while grain yield increased 6—14%. These higher yields at eCO_2, were attributed

to increased plant height and a larger flag leaf area for the eCO_2 treatments. Nutrient accumulation was split evenly pre- and post—anthesis at 47.0—60.0% for Mg, S, Fe, Zn, N, while 74—100% of Mn, Ca, P and K, were also accumulated before anthesis, and ≥ 62% Cu was accumulated post—anthesis. Higher Ca and Mg accumulation in K deficient crops was attributed to cation antagonism.

Chapter 7: General Discussion

7.1 Introduction

In this chapter, the major findings of the study are discussed in relation to the original objectives, hypotheses and relevant previous reports. A key hypothesis is that the CHI and NuHIs of spring wheat crops can be enhanced through improvements in agricultural practices implemented by the farmers, such as selection of improved genotypes by breeding. Other key management decision made by farmers includes fertiliser management. The current study has considered a combination of genotypes and fertiliser supply, under different environments (field, glasshouse and growth chambers (aCO_2 and eCO_2). Crop nutrient accumulation up to anthesis, and translocation during grain filling phase are related to major physiological processes influencing growth and yield formation (Pask 2009), as well as nutrient accumulation, partitioning and remobilisation between crop components (Hamnér et al. 2017). This chapter integrates the key findings to interpret results in relation to current knowledge. Also, knowledge gaps across chapters are summarised to suggest future research.

7.2 Crop harvest index (CHI) and physiological basis for yield

In Experiment 1 and 2 there was no relationship between grain yield and CHIs among the genotypes (Tables 3.4, 3.5, 5.1), suggesting that yields of the modern spring genotypes under N fertiliser supply in our study were not influenced by CHI differences. The CHI results reported in the current study, are consistent with the ~0.50 values reported for wheat since the 1980s (Austin et al. 1980; Gifford et al. 1984; Austin et al. 1989; Meinke et al. 1998; Austin 1999; Berry et al. 2007; Gaju et al. 2011; Dai et al. 2016). This suggests that the *Rht* genotypes studied may be also close to the observed CHI plateau (e.g. Austin 1999; Berry et al. 2007). These authors suggest that further yield increases must come from genetic enhancements that increase total biomass production, while utilising resources as efficiently as possible. The challenge is to identify crop characteristics that can increase resource use efficiency to underpin further improvements in wheat biomass yield. This is not without precedence, as it has been achieved in maize, through breeding for more erect leaves (Section 2.2). The key question is whether the same principles can be applied to wheat. Our results suggest that is possible, as the genotypes differed in LAER and maximum GLAI.

Furthermore, the plateauing of CHI indicates that the use of the theoretical upper limit of 0.62 (Austin et al. 1980) in crop models will overestimate grain yields.

The CHI reported in this study was influenced by the environment. The low CHI of ~0.42±0.03 in Experiment 2, compared with 0.50—0.55 in Experiment 1, 3 and 4, could be attributed to the high temperature around anthesis (Section 5.4.1). High temperature episodes near anthesis, have been shown to reduce grain density (Wheeler et al. 1996; Ferris et al. 1998), resulting in lower grain yields, and the subsequent lower CHI. These results imply that sowing dates for spring wheat have to be adjusted so that anthesis occurs before the periods of high, long term mean temperatures (end of December—January period; Figure 3.1 B). The sowing date of the first week of September in the current study, meant that anthesis for all the genotypes was ~960°Cd after emergence (Figure 3.6), at the beginning of December. This study confirmed the current New Zealand industry sowing date recommendations.

Our study showed that P deficiency reduced CHI by reducing canopy expansion rates through reduced (i) area of individual leaves (carbon source) and (ii) number of fertile tillers (carbon sink). This resulted in reduced surface for intercepting IPAR, and hence the low AGB and grain yield. Phosphorus deficiency affected the grain yield (grain density * TGW) more than the straw, and hence the high straw: grain ratio (Table 6.1), which resulted in low CHI. The reduction in grain yield under P deficiency due to reduced number of ears/pot, grains/ear and TGW (Table 6.2) has been reported previously (Goudriaan & de Ruiter 1983). Furthermore, the effects of P deficiency on growth and development of wheat under controlled environments have also been reported (Rodriguez et al. 1994; Rodriguez et al. 1998), and was associated with canopy developments and hence photosynthesis. Soil fertility amelioration has mostly concentrated on N, than other nutrients. The P deficiency results reported here suggests we should also be concerned about P fertility in wheat production.

The low CHI for optimum N fertiliser treatments in Experiment 2 was also explained by the high straw: grain ratio (Table 5.1). This has been reported previously (McNeal et al. 1971). In contrast, in Experiment 1 where N was mineralised throughout the growing season, the straw: grain ratios were unaffected by low N at 0.81±0.03 (Section 3.6; 3.7.5). The effects of

N and P fertiliser supply on CHI, highlight the importance of fertiliser management on wheat production, and could be used alongside breeding to increase grain yield.

The fastest pre-anthesis LAER, earlier attainment of $GLAI_{crit}$ and higher maximum GLAI for 'Discovery' (Figure 3.5, 5.1), enabled a better synchrony between time of peak radiation interception and peak radiation incidence (Figures 3.1C). This was similar to previous reports on maize (Stone et al. 1999). This, and the longer LAD above the $GLAI_{crit}$ (Section 3.3.2.1) during the grain-filling period (Figure 3.5) led to increased interception of IPAR, and higher AGB and grain yields. This meant that these crops were at or near maximum photosynthesis for longer (Richards 2000). This has been reported previously for winter wheat (Austin et al. 1980), spring wheat (Peltonen-Sainio et al. 2007) and maize (Birch et al. 2003; Birch et al. 2007) crops. Further increases in total post-anthesis photosynthesis may be obtained by selecting genotypes that reach anthesis earlier (Austin et al. 1980) and mature later, thus maintain maximum GLAI for longer (Figure 3.5) as reported previously for other cereal crops (Crosbie 1982; Richards 2000). A potential wheat ideotype (Donald 1968) should therefore have the inherent ability for faster LAER, and maintaining maximum GLAI above the $GLAI_{crit}$ for longer, e.g. 'Discovery' (Figure 3.5, 5.1). This is important as grain yield originates mainly from post-anthesis photosynthesis, when no new leaves are produced and GLAI is declining (Austin et al. 1980).

The SLN content was above the critical threshold of 0.8—1.3 g N/m^2 (Section 1.2) in Experiment 1 and 2 across the treatments. This meant SLN had no effects photosynthetic capacity and therefore RUE (Sinclair & Horie 1989). This means that the differences in AGB and grain yield were attributed to canopy development and IPARi.

The high grain yield for 'Discovery' in Experiment 1 and 2 were explained by higher TGW. These findings suggests that the incorporation of suitable traits may provide potential for the development of a wheat ideotype for the temperate maritime climates like New Zealand. An ideal wheat crop should utilise its photosynthate more efficiently (Richards 2000), as there is potential to increase yield through partitioning of the surplus carbohydrates for the initiation of more grains or larger TGW (Figure 3.4; Table 5.2). In all four experiments, the relationship between TGW and grain density (Figure 3.4; 5.1; Table 6.2), showed that the highest yielding genotype 'Discovery' was positive and above the regression line. This suggests that 'Discovery' had more total carbon in the grains than the

average of the other genotypes and was therefore consistently able to produce a higher TGW at the same grain density. These results further confirm that the relationship between TGW and grain density can be used to explain yield differences.

The eCO_2 affected yield and not CHIs (Table 6.1). This showed that yield was affected more by the changes in total production than the partitioning of photosynthate to the grain. This is important, as CHI for wheat has plateaued, thus any further yield increases must come from genetic enhancements that increase total biomass production (Richards et al. 2019), similar to maize (Russell 1991). The increase in AGB and grain yield with eCO_2, (Section 6.4.1) was consistent with recent reports (Broberg et al. 2019; Maphosa et al. 2019).

7.3. Nutrient partitioning into the grain

The NHI differences among the genotypes were small at ≤ 6.40%. This meant that NHI is less useful as a trait, in the genetic improvement of these genotypes through breeding. The low NHI for 'Discovery' and 'Duchess' in Experiment 1 and 2, was attributed to low translocation for 'Duchess', as it had a high AGN and N_g yield did not differ among the genotypes in both experiments. However, for 'Discovery', the low NHI was a result of N dilution as it had the highest biomass and grain yield. The NHI reported in this study, shows that the genotypes retained moderate amounts of N in the straw at harvest maturity. This is significant as it reduces net N export from the farm and also the residual soil N and the potential N losses into the environment. The NHI range of 0.72—0.86 across the four experiments is consistent with the 0.70—0.90 reported for modern winter wheat (Gaju et al. 2011; Gorjanović et al. 2011; Pask et al. 2012; Belete et al. 2018). This means NHI was consistently high irrespective of treatments or growing environment and also between wheat types.

The calculated GPC of 10.6—13.1% across the four experiments (Sections 3.9.3.3, 5.4.2, 6.3.6) was within the standard quality requirements (Reddecliffe et al. 2000) for milling wheat in New Zealand. The fact that some genotypes (e.g. PFR-3019 and 'Reliance') attained the milling quality requirements at low N fertiliser supply is encouraging, however the differences in GPC was small to be used as a basis for future breeding programs. Furthermore, PFR-2021 did not follow the well-known inverse relationship between yield and Ng% in Experiment 1, attributed to its high PANU compared with the other genotypes. However, we were unable to confirm this result in Experiment 2, due to bird damage

(Section 5.2.1), but is a worth trait to follow up and confirm in future. Results from Experiment 1 and 2 show that when the objective is to grow for high GPC, then 'Reliance' would be the most appropriate choice, while for high yield, but moderate GPC requirements, 'Discovery' would be the appropriate genotype.

The NuHIs reported in this study (Figure 7.1) show that the environment was important in the partitioning of individual nutrient elements, thus nutrient partitioning may not be an ideal candidate trait for future breeding purposes. Furthermore, there was no relationship (R^2 = 0.06) between NuHIs and proportion of nutrients at anthesis.

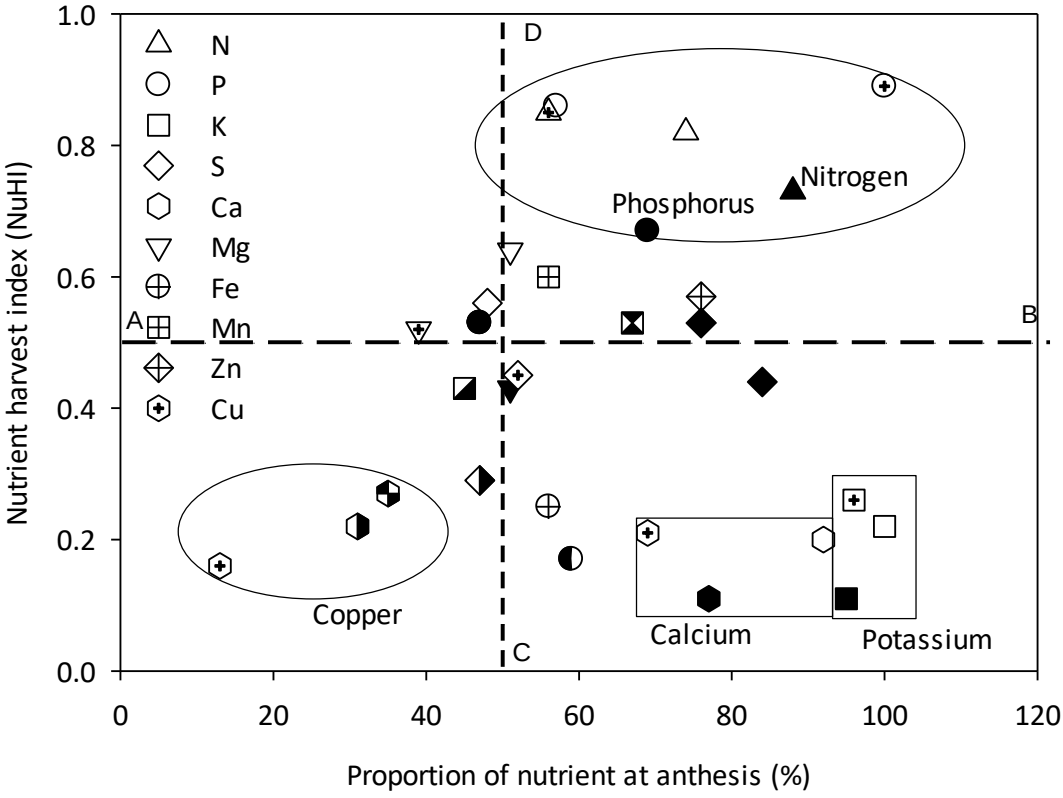

Figure 7.1: Relationship between nutrient harvest indices (NuHIs) and nutrient uptake pattern (proportion) at anthesis for spring wheat genotypes grown under different environments: Experiments 1 (open [macro-] and crossed [micro-nutrient] symbols), Experiments 2 (closed [macro-] and semi-filled [micro-nutrient] symbols) and Experiments 3 and 4 (cross-hair [macro-] and hourglass [micro-nutrient] symbols). Dotted lines represent NuHI of 0.50 (A—B) and 50% nutrient uptake (C—D)

Results also show that NuHIs are a function of remobilisation efficiency, rather than timing of uptake for the different nutrients (Figure 7.1). The high NHI and PHI across the

environments, was due to their efficient translocation from the vegetative to the grain component, as crops had taken up 60—100% of N and P at anthesis. In contrast, the low CaHI and KHI showed these nutrient elements were not readily translocated to the grain even though 70—100% of Ca and K had been accumulated by anthesis. These results are consistent with Hamnér et al. (2017). The patterns of nutrient uptake helps farmers decide timing of fertiliser application, as those taken up earlier, e.g. Ca and K, should be applied at sowing, while those that are mostly taken up after anthesis, such S and Zn, should be split applied at sowing and during vegetative growth.

7.3.1 Nitrogen use efficiency (NUE)

The NUE results have not shown which traits are important at low N conditions as all genotypes behaved the same. This was despite the fact that genotypes used in this study were recommended by wheat breeders on the basis that they had a range of attributes that could enhance NUE. Gaju et al. (2011) reported that the current genotype testing at relatively high N supply conditions, means that those selected perform well under these conditions. Subsequently, cultivars selected for high yield, under high N conditions may not be the highest yielding under low N conditions. The implication is that for breeders to develop cultivars which yield well at lower N supplies, they will require breeding and testing at low levels of N supply (Brancourt-Hulmel et al., 2003) or an understanding of which plant traits are important for yield under these conditions. Improvements in NutE has been mainly driven by CHI (Pask 2009), and therefore one of the most important aims for future breeding for lower N requirements is to increase AGB production under low N supply while maintaining the current values of CHI (Calderini et al. 1999). This can be achieved by selection for increased AGB production per unit of crop N, termed the biomass production efficiency (BPE). In the current study, the decline in NutE with increasing N supply (Tables 3.10, 5.3) was the result of a decline in BPE, whilst CHI was unaffected by N fertiliser supply in Experiment 1. There are inconsistence in the contribution of NUE components in this study, and in literature (e.g. Ortiz-Monasterio et al. 1(997), however, it is logical that NupE is more important in low N situation and NutE in high N situation as more N is readily available in the latter.

7.4 Scope for further studies

As the present study tested only a small range of spring milling wheat genotypes currently commercially available in New Zealand, these results will require confirmation with a larger range of genotypes. The differences among genotypes in CHI were small to moderate (10.0—30.0%), while small (4.0—8.0%) for NHI and therefore there is need for further experimentation with a wider germplasm to confirm these differences, and how they fit into future breeding programmes. Specifically, new sources of germplasm may be found outside the *Triticeae* (Austin 1999), for example, the wider relatives of wheat or synthetically derived wheat (Reynolds et al. 2006). Furthermore, comparisons with other cereal crops may reveal traits which may be introgressed into spring wheat genotypes, e.g. N dynamics and stay-green traits for sorghum (Borrell et al. 2001). This work can also be extended to analysing the existing genetic variability in different environments, and incorporation of more levels of N fertiliser supply, especially under low N conditions, thus increasing pressure for N efficient genotypes which sustain yield levels with less fertiliser N use.

The results suggests that there are opportunities to improve grain yield through breeding for faster LAER and higher maximum GLAI. Extending canopy investigations to more genotypes, and physiological traits, such as crop extinction coefficient could also be used to understand canopy dynamics, as reported for maize (Crosbie 1982; Stone et al. 1998; Stone et al. 1999). Results could be used to adjust sowing patterns and rates, thus timing of canopy closure, also similar to the experience in maize.

The severe impact of P deficiency on CHI and NuHIs compared with the control, highlighted the importance of P in crop production. There was not a response of CHI to CO_2 under our experimental conditions when water was not a liming factor. This agrees with literature (Gifford 1979). However, the CHI response to increasing CO_2 level (Amthor 2001) has been reported to increase with water stress or decrease with N stress (Section 6.4.1). The interaction of P fertiliser supply with water stress and N fertiliser supply has not been elucidated. A follow up experiment on effects of water, N and P fertiliser supply on spring wheat grown under eCO_2, would complete this investigation.

7.5 Conclusions

The research described in this thesis has highlighted the effects of management and canopy development on spring wheat genotypes. The study highlighted the importance of early canopy development (greater IPARi) and P fertiliser (Experiments 3 and 4) on AGB and grain yield. Consequently, the following conclusions can be drawn from this study:

- Differences in grain yield in Experiments 1 and 2, showed that genotype differences existed, particularly under optimum N conditions, with 'Discovery' consistently producing higher grain yield compared with 'Reliance'. The faster pre-anthesis LAER, earlier attainment of $GLAI_{crit}$ and higher maximum GLAI for 'Discovery', coupled with a longer LAD above the $GLAI_{crit}$ during the grain-filling period led to increased interception of IPAR, and higher AGB and grain yields.
- The SLN content for all genotypes in Experiment 1 and 2 were above the critical threshold of 1.1 (0.8—1.3) g N/m^2. This meant SLN did not affect the photosynthetic capacity and therefore RUE. Therefore the differences in AGB and grain yield reported in this study were attributed to canopy development and solar radiation interception.
- In Experiment 1 and 2, CHI was independent of grain yield, and 'Duchess' had a lower CHI than the average of the other genotypes. In Experiment 3 and 4, P deficiency reduced CHI by reducing canopy expansion rates through reduced area of individual leaves (carbon source) and number of fertile tillers (carbon sink).
- The relationship between TGW and grain density, showed that genotypes with TGW located positively above the regression line produced more grain yield, e.g. 'Discovery' in Experiment 1 and 2. The implication is that TGW can be used as a discriminator of total carbon captured and allocated to the grain. These results further confirm that the relationship between TGW and grain density can be used to explain yield differences, and can be used for future breeding selections.
- In Experiment 1 and 2, the NHI differences among the genotypes were small (≤ 6.40%), and NHIs were lower for 'Discovery' and 'Duchess', compared with the other genotypes. The NHI was severely reduced (37%) by P deficiency compared with the fertilised crops.

- The NuHIs differed among the genotype across the experiments, but were inconsistent among genotype under the different environments. There was no relationship between NuHIs and the proportion of nutrients at anthesis and therefore individual NuHIs were a function of remobilisation efficiency, rather than timing of uptake.
- The high NHI and PHI across the environments, was due to their efficient translocation from the vegetative to the grain component, while the low CaHI and KHI showed these nutrient elements were not readily translocated to the grain, as 60—100% of Ca, K, N and P had been accumulated at anthesis.
- Similar NUE at low N shows that the selected genotypes had no differentiating traits that could be used for breeding, specific to low N fertility conditions. This was despite fact that genotypes used in this study were recommended by wheat breeders on the basis that they had a range of attributes that could enhance NUE.
- In Experiment 3 and 4, CO_2 level had little effects on CHI, NHI and NuHIs. These relatively small changes were associated with an increase in growth.
- Biomass and grain yield increased with eCO_2, attributed to increased plant height and a larger flag leaf area.

These results support the hypothesis that active selection for canopy characteristics that confer rapid early leaf growth, particularly during stem extension, can lead to increased grain yields under these environments. The high grain yield for 'Discovery' was explained by higher TGW. These findings suggests that the incorporation of suitable traits may provide potential for the development of a wheat ideotypes able to utilise photosynthate more efficiently, as there is potential to increase yield through partitioning of the surplus carbohydrates for the initiation of more grains or larger TGW.

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Appendix 1.1: Genotype background, agronomic and quality information for the six genotypes used in the experiments (FAR 2018; PGG-Wrightsons 2018) and details for the numbered genotypes are from Plant and Food Research Limited (PFR) (Hay 2014; FAR 2018; P. Johnston, pers. comm)

Characteristics	Duchess	PFR-3026	PFR-3019	Discovery	Reliance	PFR-2021
Parentage	Conquest*Option	Saracen*CFR00-540-89-3-4	CFR02-244.3 *Conquest	Tybalt*Morph	Conquest*Option	5870.7.4.5.9*CFR00-878-41-1-4
Breeder[1]	PFR	PFR	PFR	Limagrain (UK)	PFR	PFR
Year released	2013	NA	NA	2014	2013	NA
Agronomic characteristics	Facultative, awnless	Facultative, awned	Facultative, awned	Facultative, awnless	Facultative, awned	Facultative, awned
Grain yield (85% DW, t/ha)	8.80	9.70	9.00	10.3	10.2	8.00
Endosperm texture	Hard	Hard	Hard	Hard	Hard	Hard
Grain protein (%)	12.2	12.5	12.9	11.4	13.2	11.3
NHI	0.77	0.84	0.84	0.81	0.85	0.83
TGW (g)	44.7	45.8	50.8	53.8	46.5	48.0
Test weight (kg/hl)	76.3	81.1	80.7	76.5	75.5	79.2
Screenings (%)	1.80	0.70	0.66	0.77	1.17	1.10
Falling numbers (sec)	382	-[2]	-	357	384	-
Resistance to lodging	Resistant	Resistant	Resistant	Moderately susceptible	Resistant	Resistant
Height without PGR	1.00-1.10 m	0.90-0.95 m	0.95-1.00 m	1.20-1.40 m	0.90-0.95 m	0.98-1.15 m

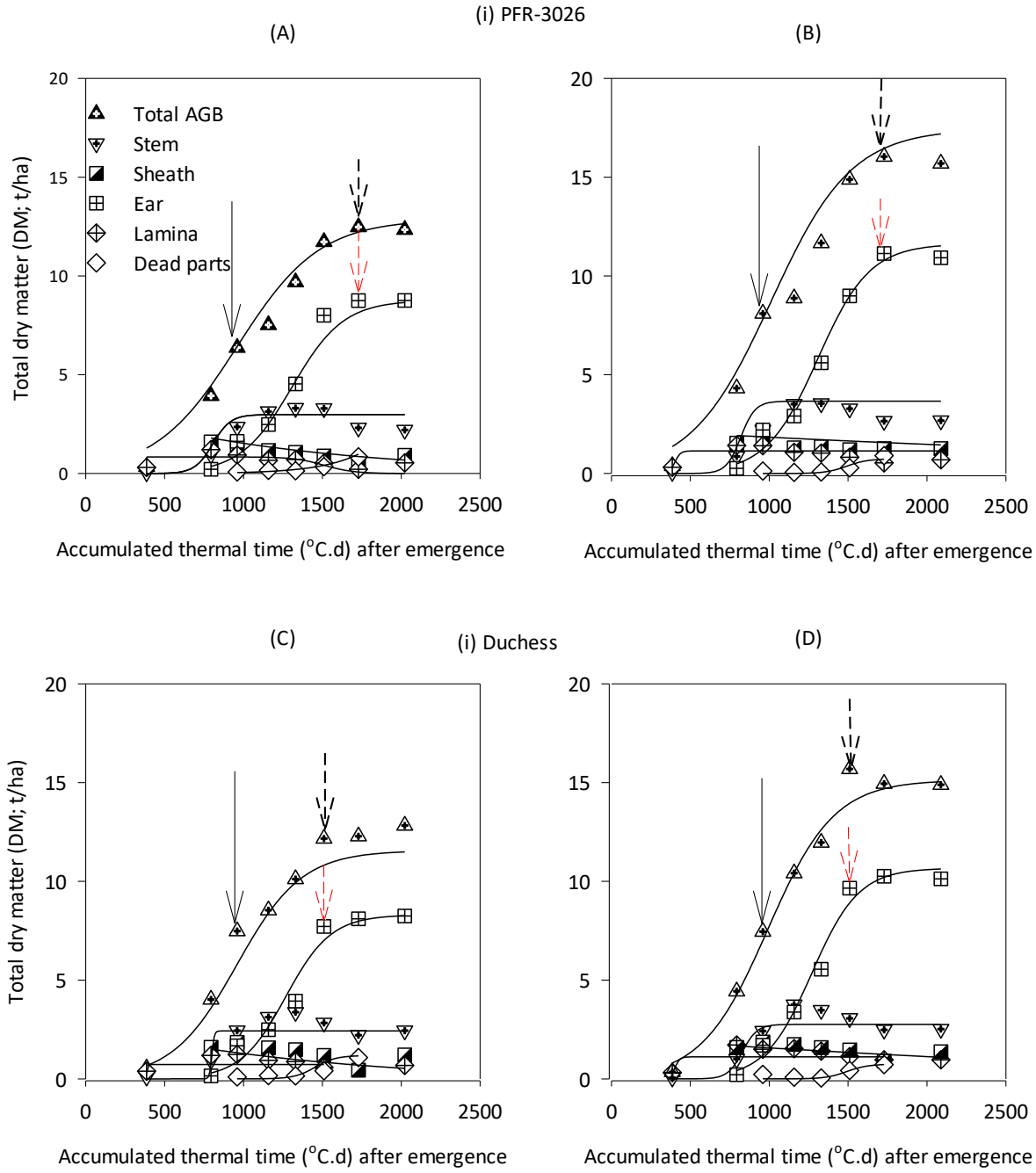
[1] PFR = Plant and food Research Limited, New Zealand, [2]No available data

Appendix 2.1 Functions of elements in higher plants, for more details refer to the following publication (McLaren & Cameron 1996; Mengel et al. 2001; Epstein & Bloom 2005)

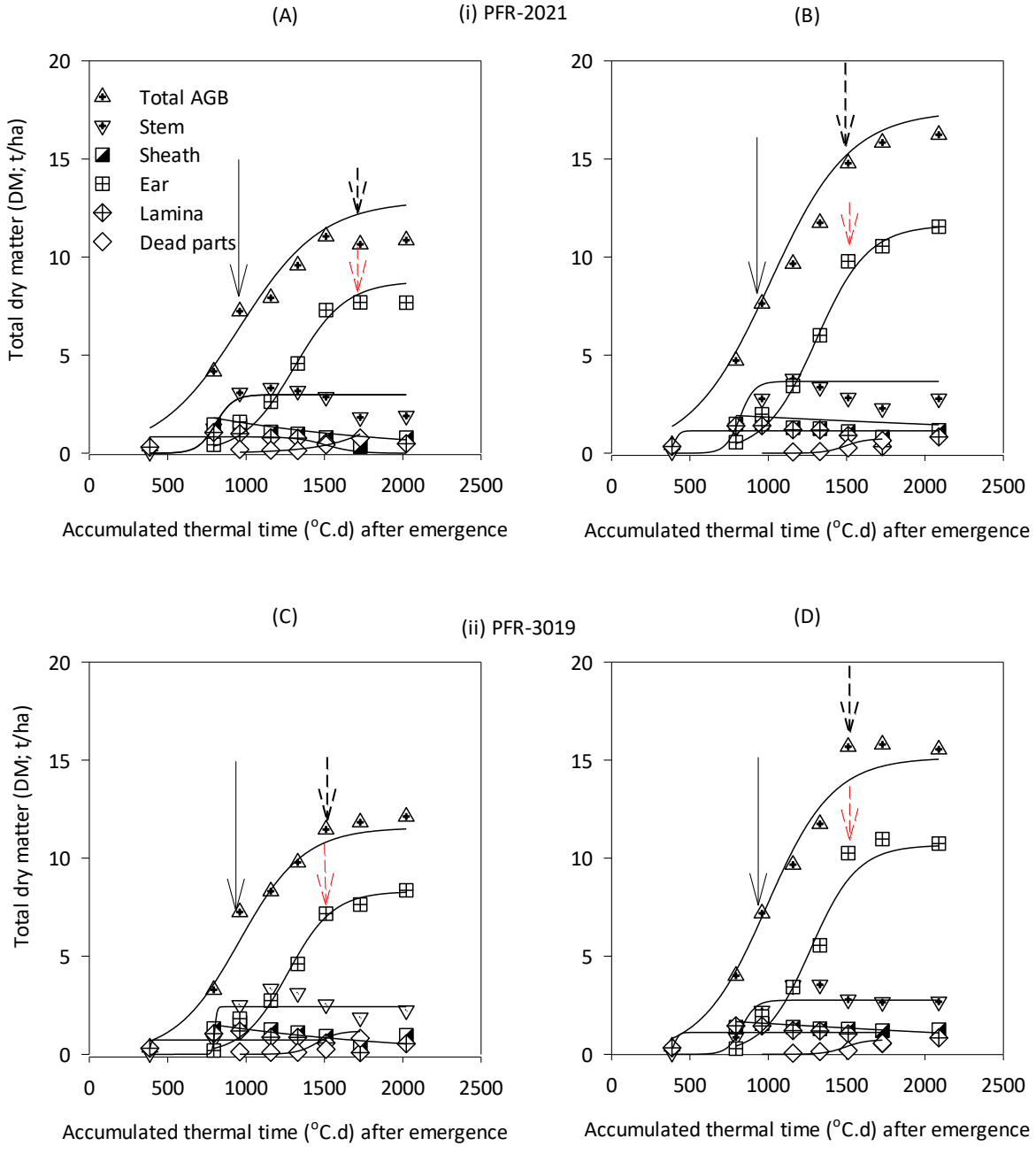
Element	Typical concentration	Physiological process	Activator of enzymes	Constituent of metabolite or cell component	Deficiency symptoms	Phloem mobility
Macro-	**%DM**					
N	2.0-4.0	• photosynthesis		• Amino acids, proteins, nucleic acids, nucleotides, chlorophyll	• Chlorosis, starting with older leaves • Reddening in cold weather	mobile
P	0.10-0.40	• Energy storage and transfer • Membrane integrity		• ATP, nucleotides, nucleic acids, phospholipids	• Dark green foliage • Reddening or purpling of leaves or petioles. Older leaves first	mobile
K	1.0-3.0	• Translocation, water and energy relations, stomatal opening. • regulation of cellular pH • Osmoregulation • cation—anion balance	+		• Marginal chlorosis and necrosis • Red pigmentation on young leaves	mobile
Ca	0.50-3.0	• Membrane maintenance, cell division and regulation • cell wall stabilisation • Osmoregulation • cation—anion balance	+	• calcium pectates	• Growing tips die • Fruit disorders e.g. blossom end rot in tomatoes • leaf tip burn	immobile

Mg	0.10-0.50	• CO_2 regulation • regulation of cellular pH • cation—anion balance • protein synthesis and carbohydrate partitioning	+	• chlorophyll, ribisomes	• Marginal or interveinal chlorosis; older leaves	mobile
S	0.20-0.50	• Protein synthesis and functions • Energy storage and transfer		• Amino acids, coenzymes, ferredoxins, sulfolipids, proteins	• Chlorosis of whole plant, young leaves first	variable mobility
Micro-	**mg/kg DM**					
Zn	20-100	• Auxin metabolism, nucleotide synthesis, membrane integrity	+	• Dehydrogenases, CuZn superoxide dismutase, carbonic anhydrase, RNA polymerase, alkaline phosphatase, phospholipase	• little leaf and rosetting • chlorotic mottle in less severe cases	variable mobility
Fe	50-300	• Oxidation-reduction in electron transfer		• Iron porphyrins (leaves), ferredoxins	• Interveinal chlorosis, occurs first in young leaves	immobile
Mn	20-250	• Oxidation-reduction in electron transfer • O_2 evolution in photosynthesis	+	• Mn superoxide dismutase	• Interveinal chlorosis, when severe, occurs first in middle leaves	immobile
Cu	5.0-15	• Lignin synthesis, terminal oxidation in redox reactions • Pollen formation and fertilisation		• Ascobate, phenyl and cytochrome oxidase • CuZn superoxide dismutase, • plastocyanini	• Death of young leaves, chlorosis and • failure of fertilisation and fruit set	variable mobility

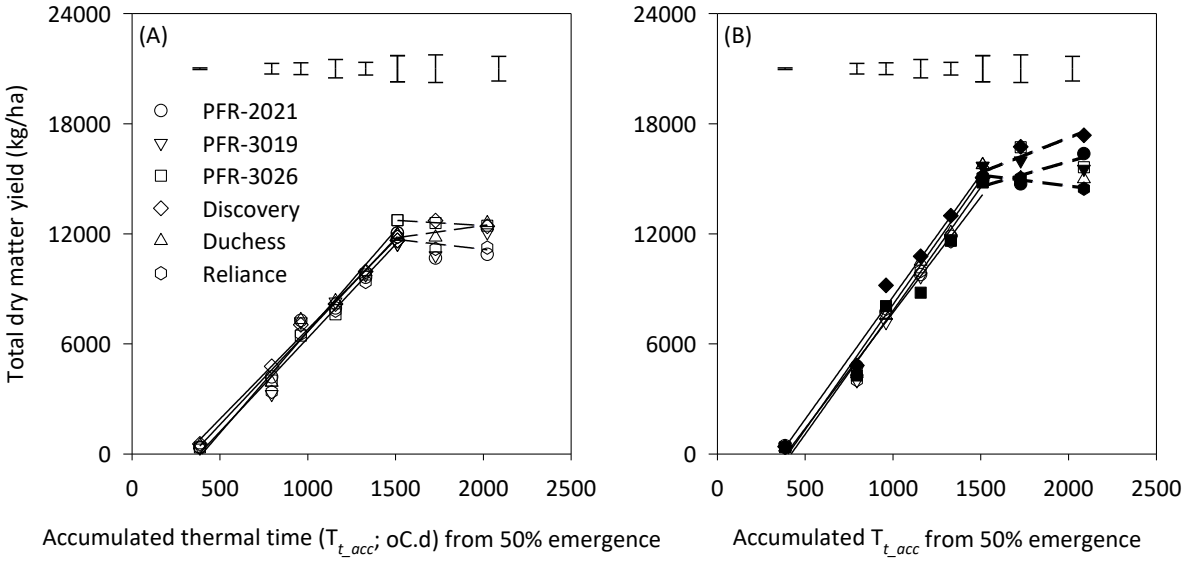
Appendix 3.1a: Relationship between mean accumulated biomass (t/ha) for the different plant organs (partitions) (see Key in Figure 3.1 A) and accumulated thermal time ($Tt_{acc,}$ °Cd) for the high yield genotype, PFR-3026 (A & B) and 'Duchess' (C & D), grown with 0 kg N/ha (A & C) and 200 kg N/ha (B & D), at Lincoln, Canterbury, New Zealand (Experiment 1). The solid and dotted arrows [black, total AGB and red, ear biomass] show anthesis and the physiological maturity (GS86), respectively.



Appendix 3.1b: Relationship between mean accumulated biomass (t/ha) for the different plant organs (see Key in Figure 3.1 A) and accumulated thermal time ($Tt_{acc,}$ °Cd) for the high yield genotype, PFR-2021 (A & B) and PFR-3019 (C & D), grown with 0 kg N/ha (A & C) and 200 kg N/ha (B & D), at Lincoln, Canterbury, New Zealand (Experiment 1). The solid and dotted arrows [black, total AGB and red, ear biomass] show anthesis and the physiological maturity (GS86), respectively.

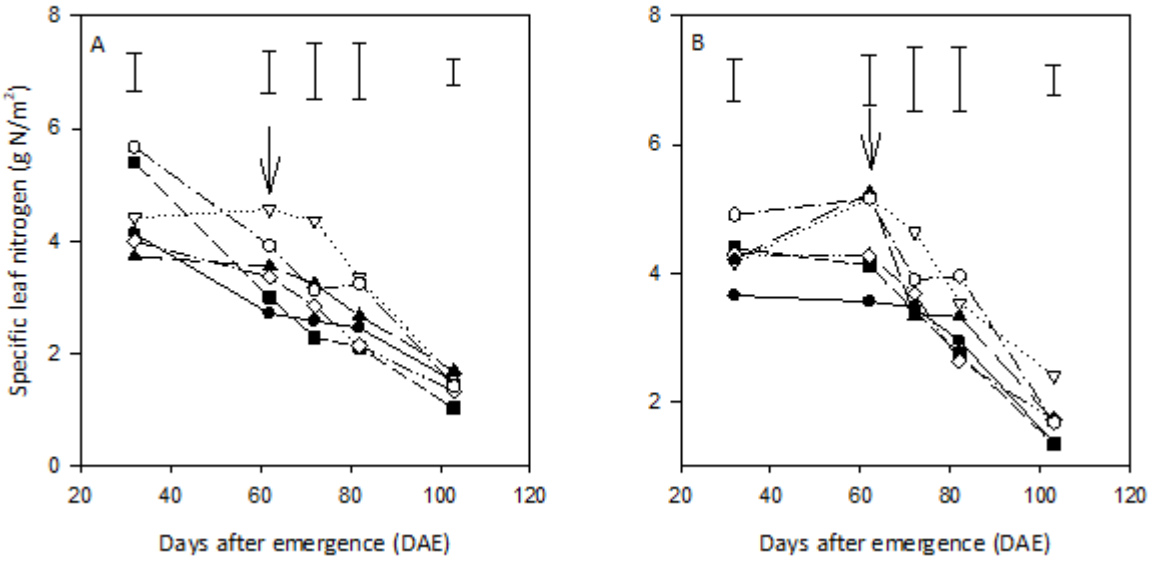


Appendix 3.2: Accumulated dry matter (kg/ha) for the different N fertiliser treatments: (A) 0 kg N/ha and (B) 200 kg N/ha and wheat genotypes grown at Lincoln, New Zealand, during 2017-18 season (Experiment 1). Vertical bars are the least significant differences ($LSD_{5\%}$). Coefficient values of each regression (solid lines) are in Appendix Table 3.1.

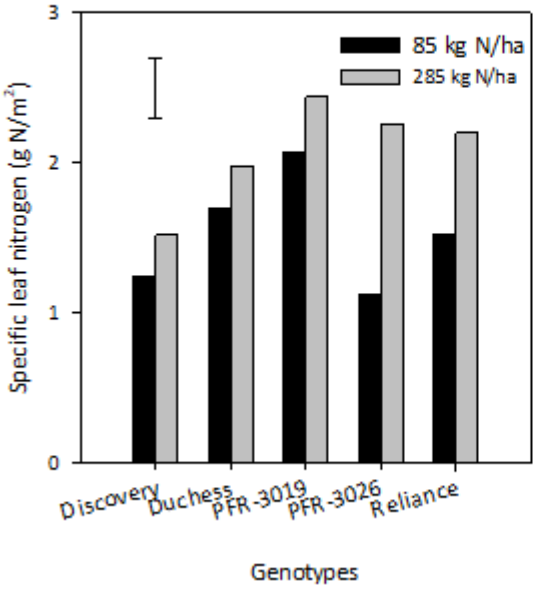


Appendix 3.3: Specific leaf N (g/m^2) for different wheat genotypes (● PFR-2021, ▼ PFR-3019, ■ PFR-3026, ◆ 'Discovery', ▲'Duchess' and ⬣ 'Reliance') grown at low (A) and optimum (B) N fertiliser supply in Experiment 1 and at anthesis in Experiment 2. Vertical lines in Experiment 1 and 2 represent $LSD_{5\%}$, and the solid arrows in Experiment 1 are the anthesis point.

Experiment 1



Experiment 2



Appendix 3.4: Equations and coefficients of determination (R^2) for regression fitted to data in Appendix 3.2.

Genotype	N treatments (kg/ha)		
	0	200	Mean
	Equation (Y=)		
PFR-2021	10.2x – 3476 (R^2= 0.98)	11.9x – 4801 (R^2= 0.99)	11.0x – 3536 (R^2= 0.99)
PFR-3019	10.7x – 3703 (R^2= 0.97)	12.1x – 5655 (R^2= 0.98)	11.4x – 4111 (R^2= 0.98)
PFR-3026	10.6x – 4118 (R^2= 0.99)	11.8x – 4918 (R^2= 0.98)	11.2x – 3901 (R^2= 0.98)
'Discovery'	10.4x – 2979 (R^2= 0.99)	12.2x – 4780 (R^2= 0.98)	11.3x – 3630 (R^2= 0.99)
'Duchess'	9.21x – 3509 (R^2= 0.99)	13.1x – 5483 (R^2= 0.99)	11.1x – 2955 (R^2= 0.99)
'Reliance'	10.7x – 3884 (R^2= 0.99)	12.5x – 5240 (R2= 0.99)	11.6x – 3857 (R^2= 0.98)
Mean	10.3x – 3612b (R^2= 0.98)	12.3x – 5146_a (R^2= 0.98)	11.3x – 3665 (R^2= 0.98)

Appendix 4.1: Relationship between nutrients and nitrogen (N) concentration for shoots at anthesis and harvest maturity for six wheat genotypes grown at low and optimum N fertiliser supply (Experiment 1).

Element	Anthesis	Harvest maturity
Phosphorus	0.05x + 0.13; R^2 = 0.72	0.01x + 0.24; R^2 = 0.003
Potassium	0.41x + 0.78; R^2 = 0.58	0.55x + 0.11; R^2 = 0.37
Sulphur	0.12x – 0.04; R^2 = 0.89	0.20x – 0.10; R^2 = 0.54
Calcium	0.14x – 0.03; R^2 = 0.74	0.16x – 0.08; R^2 = 0.47
Magnesium	0.05x + 0.01; R^2 = 0.81	0.04x + 0.05; R^2 = 0.19
Iron	14.4x + 63.0; R^2 = 0.10	5.87x + 83.0; R^2 = 0.01
Manganese	7.44x + 38.1; R^2 = 0.10	6.01x + 31.0; R^2 = 0.03
Zinc	-2.71x + 36.1; R^2 = 0.02	10.8x + 13.5; R^2 = 0.22
Copper	1.86x + 1.37; R^2 = 0.74	2.49x + 19.1; R^2 = 0.01

Appendix 4.2: Grain N: nutrient ratio for five wheat genotypes grown at low and high N fertiliser supply at Lincoln, New Zealand in 2018-19 season (Experiment 2).

Treatments	N/P	N/K	N/S	N/Ca	N/Mg	N/Fe	N/Mn	N/Zn	N/Cu
85 kg N/ha									
PFR-2021	6.05_{a}[1]	6.28_{a}	10.9_{a}	42.8_{bc}	18.0_{a}	550_{a}	509_{ab}	778_{a}	3270_{a}
PFR-3019	5.76_{a}	6.14_{a}	10.5_{a}	40.7_{bc}	15.4_{b}	482_{b}	474_{b}	670_{b}	3231_{a}
PFR-3026	4.89_{a}	4.73_{b}	10.7_{a}	38.4_{bc}	16.4_{b}	476_{b}	452_{b}	687_{b}	3363_{a}
Discovery	5.64_{a}	4.91_{b}	10.0_{a}	43.8_{b}	17.8_{a}	536_{a}	581_{a}	803_{a}	3514_{a}
Duchess	5.45_{a}	6.36_{a}	10.0_{a}	36.2_{c}	16.9_{a}	540_{a}	456_{b}	727_{ab}	3111_{a}
Reliance	5.43_{a}	5.21_{b}	10.3_{a}	57.9_{a}	15.1_{b}	487_{b}	562_{a}	670_{b}	3157_{a}
Mean	*5.54_{b}*	*5.61_{b}*	*10.4_{b}*	*43.3_{a}*	*16.6_{b}*	*512_{b}*	*506_{b}*	*723_{a}*	*3274_{a}*
285 kg N/ha									
PFR-2021	6.15_{a}	6.70_{a}	10.6_{a}	46.4_{ab}	17.2_{b}	597_{ab}	586_{ab}	705_{ab}	2839_{b}
PFR-3019	6.37_{a}	6.02_{c}	10.7_{a}	40.5_{bc}	16.2_{b}	533_{b}	523_{b}	649_{b}	3360_{a}
PFR-3026	6.04_{a}	6.21_{bc}	10.9_{a}	35.2_{c}	17.7_{ab}	545_{ab}	530_{b}	693_{ab}	3988_{a}
Discovery	6.95_{a}	6.12_{bc}	10.9_{a}	46.5_{ab}	19.1_{a}	545_{b}	629_{a}	720_{ab}	3443_{a}
Duchess	6.87_{a}	8.33_{a}	10.8_{a}	35.7_{c}	19.6_{a}	621_{a}	508_{b}	722_{a}	3955_{a}
Reliance	6.91_{a}	6.99_{b}	10.8_{a}	50.0_{a}	18.7_{ab}	576_{ab}	623_{a}	690_{ab}	3541_{a}
Mean	*6.55_{a}*	*6.73_{a}*	*10.8_{a}*	*42.4_{a}*	*18.1_{a}*	*569_{a}*	*566_{a}*	*697_{a}*	*3521_{a}*
Significance: P value (LSD5%)									
N fertiliser	<0.001(0.35)	<0.001 (0.52)	0.05 (0.39)	0.63 (3.92)	0.002 (0.88)	0.001 (32.7)	0.02 (50.2)	0. 20 (41.1)	0.20 (383)
Genotype (G)	0.12 (0.61)	0.003 (0.90)	0.75 (0.68)	<0.001 (6.79)	0.02 (1.53)	0.06 (56.6)	0.02 (87.0)	0.05 (71.1)	0.49 (664)
N*G	0.17 (0.86)	0.16 (1.28)	0.53 (0.96)	0.54 (9.61)	0.12 (2.17)	0.74 (80.1)	1.0 (123)	0.57 (101)	0.41 (938)

[1]Means with letter subscripts are compared within each column, and those with the same letters not different at $\alpha = 0.05$.

Appendix 5.1: Nutrient accumulation at anthesis for five wheat genotypes grown at low and optimum N fertiliser supply in a Glasshouse (Experiment 2) at Lincoln, New Zealand in 2018-19 season. See Figure 5.7; 5.8.

Treatments	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu
85 kg N/ha									
Discovery	18.1_{a}[1]	134_{a}	8.47_{a}	11.5_{a}	7.17_{a}	0.69_{b}	0.71_{a}	0.20_{a}	0.07_{a}
Duchess	15.3_{a}	128_{a}	6.76_{a}	10.2_{a}	6.85_{a}	0.73_{b}	0.61_{a}	0.30_{a}	0.07_{a}
PFR-3019	11.2_{a}	127_{a}	5.53_{a}	8.30_{a}	4.52_{a}	1.84_{a}	0.36_{a}	0.22_{a}	0.12_{a}
PFR-3026	15.3_{a}	134_{a}	8.14_{a}	10.4_{a}	7.11_{a}	0.99_{b}	0.67_{a}	0.27_{a}	0.09_{a}
Reliance	16.6_{a}	140_{a}	8.35_{a}	11.8_{a}	6.73_{a}	0.88_{b}	0.74_{a}	0.18_{a}	0.07_{a}
Mean	*15.3_{b}*	*133_{b}*	*7.45_{b}*	*10.4_{b}*	*6.48_{b}*	*1.03_{b}*	*0.62_{b}*	*0.24_{b}*	*0.08_{b}*
285 kg N/ha									
Discovery	126_{a}	867_{a}	77.3_{a}	59.4_{a}	37.9_{a}	3.22_{a}	1.76_{a}	0.96_{b}	0.30_{a}
Duchess	98.5_{b}	898_{a}	71.3_{a}	40.7_{b}	32.3_{b}	3.60_{a}	1.39_{b}	1.54_{a}	0.29_{a}
PFR-3019	67.0_{d}	533_{c}	40.6_{c}	20.5_{c}	21.4_{d}	2.20_{c}	0.61_{e}	0.69_{c}	0.19_{c}
PFR-3026	67.5_{d}	679_{b}	54.1_{b}	33.7_{b}	26.3_{c}	3.14_{b}	0.94_{d}	0.82_{b}	0.25_{a}
Reliance	80.8_{c}	619_{b}	57.8_{b}	35.6_{b}	27.1_{c}	2.67_{c}	1.16_{c}	0.73_{bc}	0.26_{a}
Mean	*87.9_{a}*	*719_{a}*	*60.2_{a}*	*38.0_{a}*	*29.0_{a}*	*2.97_{a}*	*1.17_{a}*	*0.95_{a}*	*0.26_{a}*
Significance: P value (LSD5%)									
N fertiliser	<0.001 6.74)	<0.001 (49.5)	<0.001 (5.18)	<0.001 (3.10)	<0.001 (2.56)	<0.001 (0.25)	<0.001 (0.13)	<0.001 (0.10)	<0.001 (0.02)
Genotype (G)	<0.001 (10.7)	<0.001 (78.3)	<0.001 (8.20)	<0.001 (4.90)	0.002 (4.04)	0.36 (0.40)	<0.001 (0.21)	<0.001 (0.15)	0.35 (0.05)
N*G	<0.001 (15.1)	<0.001 (111)	0.002 (11.6)	<0.001 (6.92)	0.02 (5.72)	<0.001 (0.56)	0.002 (0.29)	<0.001 (0.21)	<0.001 (0.05)

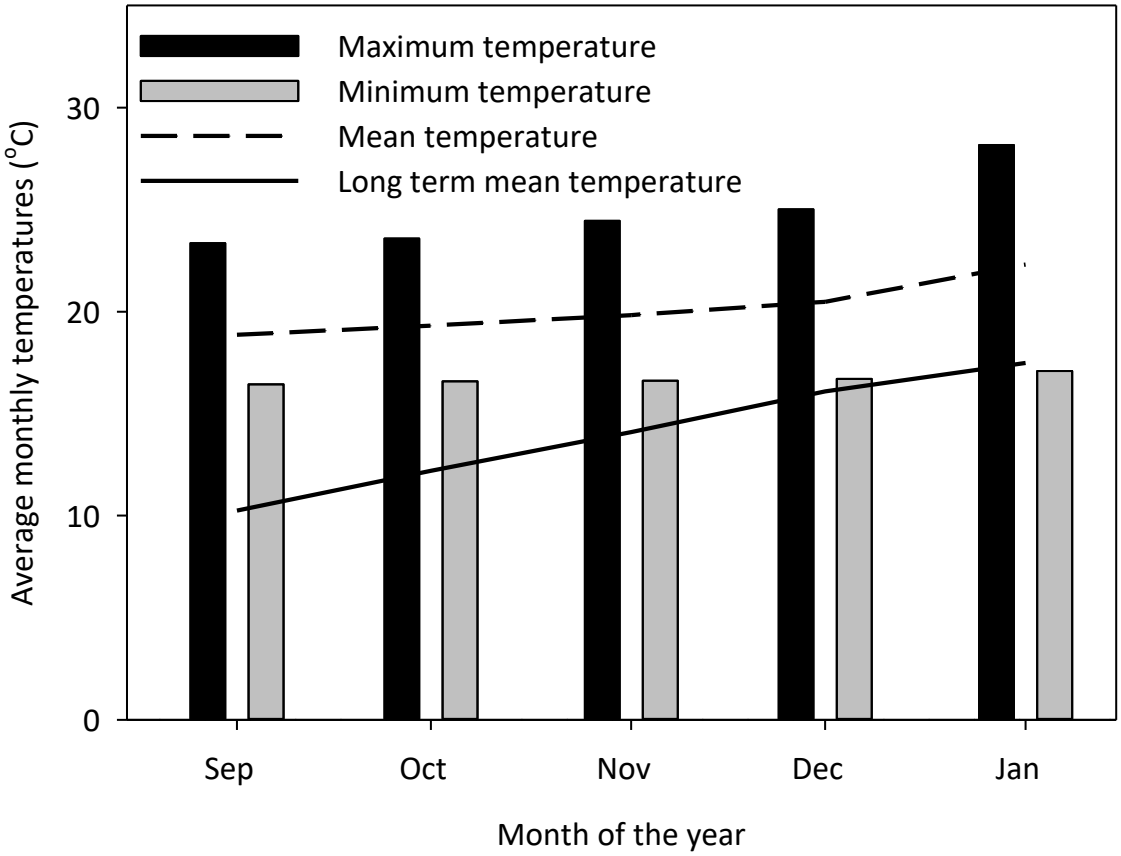
[1]Means with letter subscripts are compared within each column, and those with the same letters not different at $\alpha = 0.05$.

Appendix 5.2: Nutrient harvest index (NuHI) for five wheat genotypes grown at low and optimum N fertiliser supply in a Glasshouse (Experiment 2) at Lincoln, New Zealand in 2018-19 season (Figure 5.7; 5.8).

Treatments	PHI	KHI	SHI	CaHI	MgHI	FeHI	MnHI	ZnHI	CuHI
85 kg N/ha									
Discovery	0.71_{a}[1]	0.21_{a}	0.53_{b}	0.15_{a}	0.53_{a}	0.21_{a}	0.32_{b}	0.40_{a}	0.63_{a}
Duchess	0.63_{b}	0.17_{b}	0.58_{a}	0.08_{b}	0.43_{a}	0.29_{a}	0.20_{a}	0.45_{a}	0.46_{b}
PFR-3019	0.73_{a}	0.11_{c}	0.59_{a}	0.16_{a}	0.52_{a}	0.16_{a}	0.39_{a}	0.63_{a}	0.24_{c}
PFR-3026	0.70_{a}	0.16_{b}	0.54_{b}	0.15_{a}	0.48_{a}	0.16_{a}	0.26_{c}	0.34_{a}	0.11_{d}
Reliance	0.64_{b}	0.10_{c}	0.53_{b}	0.12_{ab}	0.48_{a}	0.21_{a}	0.25_{c}	0.36_{a}	0.10_{d}
Mean	*0.67_{a}*	*0.15_{a}*	*0.55_{a}*	*0.13_{a}*	*0.48_{a}*	*0.21_{a}*	*0.27_{a}*	*0.43_{a}*	*0.30_{b}*
285 kg N/ha									
Discovery	0.60_{abc}	0.11_{a}	0.30_{c}	0.09_{ab}	0.36_{a}	0.12_{a}	0.24_{c}	0.39_{a}	0.20_{a}
Duchess	0.60_{c}	0.10_{a}	0.35_{b}	0.05_{b}	0.34_{a}	0.13_{a}	0.21_{c}	0.47_{a}	0.16_{a}
PFR-3019	0.76_{a}	0.06_{a}	0.45_{a}	0.11_{a}	0.39_{a}	0.13_{a}	0.48_{a}	0.39_{a}	0.16_{a}
PFR-3026	0.69_{b}	0.09_{ab}	0.37_{b}	0.12_{a}	0.43_{a}	0.14_{a}	0.32_{b}	0.50_{a}	0.10_{a}
Reliance	0.64_{b}	0.07_{b}	0.32_{bc}	0.08_{a}	0.37_{a}	0.12_{a}	0.25_{c}	0.39_{a}	0.14_{a}
Mean	*0.66_{a}*	*0.09_{b}*	*0.36_{b}*	*0.11_{b}*	*0.38_{b}*	*0.13_{b}*	*0.30_{a}*	*0.43_{a}*	*0.15_{a}*
Significance: P value (LSD5%)									
N fertiliser	0.36 (0.04)	<0.001 (0.02)	<0.001 (0.03)	0.003 (0.02)	0.002 (0.06)	0.002 (0.04)	0.18 (0.03)	0. 83 (0.08)	<0.001 (0.06)
Genotype (G)	0.003 (0.06)	<0.001 (0.03)	<0.001 (0.04)	0.001 (0.05)	0.53 (0.09)	0.27 (0.07)	<0.001 (0.04)	0.27 (0.12)	<0.001 (0.10)
N*G	0.43 (0.09)	0.11 (0.04)	0.25 (0.06)	0.83 (0.07)	0.73 (0.13)	0.24 (0.10)	0.01 (0.06)	0.10 (0.17)	<0.001 (0.14)

[1]Means with letter subscripts are compared within each column, and those with the same letters not different at $\alpha = 0.05$.

Appendix 5.3: Monthly average maximum, minimum and mean temperatures (°C) in the Glasshouse (September 2018 to January 2019) and long-term mean (LTM) data at Lincoln, Canterbury, New Zealand. Long-term data are from 1970 to 2010 (NIWA 2019).



Appendix 6.1: Shoot nutrient concentration (N, S, Ca, Mg, Fe, Mn, Zn and Cu) at anthesis and harvest maturity for wheat (cv. Discovery) grown with deficient P and K fertiliser supply (Fert.) at elevated carbon-dioxide (eCO_2) in Experiments 3 and 4, at Lincoln, New Zealand in 2019-20 growing season. [1]Means with letter subscripts in common are not significantly different at α = 0.05

			Anthesis[1]								Harvest maturity							
Exp	Treatment		N	S	Ca	Mg	Fe	Mn	Zn	Cu	N	S	Ca	Mg	Fe	Mn	Zn	Cu
	CO2 level	Fert. rate	%				mg/kg DM				%				mg/kg DM			
3	Ambient	Control	1.03_{b}	0.28_{b}	0.09_{b}	0.09_{c}	77.2_{b}	32.6_{b}	91.5_{a}	4.07_{b}	0.91_{a}	0.09_{a}	0.10_{b}	0.13_{b}	123_{b}	25.6_{b}	17.5_{b}	9.89_{a}
		K deficient	1.15_{b}	0.26_{b}	0.11_{b}	0.11_{b}	90.3_{b}	32.5_{b}	113_{a}	4.43_{b}	0.90_{a}	0.08_{a}	0.13_{b}	0.14_{b}	127_{a}	28.9_{b}	17.4_{b}	10.0_{a}
		P deficient	2.35_{a}	0.30_{a}	0.19_{a}	0.19_{a}	277_{a}	65.3_{a}	49.8_{a}	24.9_{a}	0.90_{a}	0.09_{a}	0.22_{a}	0.18_{a}	286_{a}	47.4_{a}	41.2_{a}	17.1_{a}
		K*P deficient	2.35_{a}	0.30_{a}	0.20_{a}	0.20_{a}	320_{a}	65.8_{a}	108_{a}	38.8_{a}	0.84_{a}	0.09_{a}	0.27_{a}	0.19_{a}	226_{a}	47.9_{a}	58.2_{a}	22.3_{a}
	Mean		*1.72_{A}*	*0.28_{B}*	*0.14_{A}*	*0.15_{A}*	191_{B}	49.0_{A}	90.7_{A}	18.1_{A}	*0.89_{A}*	*0.09_{A}*	*0.18_{A}*	*0.16_{A}*	*190_{A}*	*37.4_{A}*	*33.6_{A}*	*14.8_{A}*
	Elevated	Control	1.19_{b}	0.34_{a}	0.09_{b}	0.09_{c}	145_{b}	37.3_{b}	72.5_{a}	4.12_{b}	0.74_{a}	0.08_{b}	0.09_{b}	0.11_{c}	163_{b}	32.4_{b}	17.0_{b}	11.7_{a}
		K deficient	1.23_{b}	0.30_{b}	0.12_{b}	0.13_{b}	152_{b}	34.7_{b}	70.1_{a}	5.53_{b}	0.83_{a}	0.07_{b}	0.13_{b}	0.14_{b}	175_{b}	35.1_{b}	35.6_{ab}	12.6_{a}
		P deficient	2.09_{a}	0.32_{a}	0.20_{a}	0.20_{a}	362_{a}	65.4_{a}	93.4_{a}	29.1_{a}	0.93_{a}	0.10_{a}	0.20_{a}	0.17_{a}	293_{a}	50.8_{a}	54.9_{a}	23.6_{a}
		K*P deficient	2.20_{a}	0.30_{b}	0.20_{a}	0.20_{a}	374_{a}	64.1_{a}	38.7_{a}	28.6_{a}	0.89_{a}	0.09_{a}	0.24_{a}	0.18_{a}	376_{a}	53.4_{a}	49.2_{a}	24.1_{a}
	Mean		*1.68_{A}*	*0.31_{A}*	*0.15_{A}*	*0.16_{A}*	258_{A}	50.4_{A}	68.7_{B}	16.8_{A}	*0.85_{B}*	*0.08_{A}*	*0.16_{A}*	*0.15_{A}*	*252_{A}*	*42.9_{A}*	*39.2_{A}*	*18.0_{A}*
4	Ambient	Control	1.01_{b}	0.30_{a}	0.09_{b}	0.09_{b}	139_{c}	32.7_{b}	41.4_{a}	10.2_{b}	0.87_{a}	0.08_{b}	0.09_{d}	0.13_{c}	151_{b}	26.3_{b}	24.2_{a}	12.1_{a}
		K deficient	1.04_{b}	0.26_{b}	0.11_{b}	0.11_{b}	176_{c}	34.1_{b}	40.9_{a}	12.8_{b}	0.95_{a}	0.07_{b}	0.14_{c}	0.15_{b}	128_{b}	36.1_{b}	22.7_{a}	10.7_{a}
		P deficient	2.28_{a}	0.31_{a}	0.21_{a}	0.20_{a}	333_{b}	70.7_{a}	93.5_{a}	29.8_{a}	0.81_{a}	0.07_{b}	0.17_{b}	0.16_{b}	346_{a}	54.0_{a}	62.7_{b}	29.1_{a}
		K*P deficient	2.22_{a}	0.30_{a}	0.22_{a}	0.22_{a}	414_{a}	63.9_{a}	53.9_{a}	40.2_{a}	0.86_{a}	0.09_{a}	0.25_{a}	0.21_{a}	317_{a}	56.0_{a}	63.7_{a}	19.0_{a}
	Mean		*1.64_{A}*	*0.29_{B}*	*0.16_{A}*	*016_{A}*	266_{A}	50.4_{A}	57.4_{B}	23.3_{A}	*0.87_{A}*	*0.08_{A}*	*0.16_{A}*	*0.16_{A}*	*236_{A}*	*43.1_{A}*	*43.3_{A}*	*25.2_{A}*
	Elevated	Control	0.94_{b}	0.29_{b}	0.09_{b}	0.09_{b}	153_{c}	37.9_{b}	55.4_{a}	11.2_{b}	0.83_{a}	0.08_{a}	0.11_{b}	0.10_{b}	245_{b}	36.2_{b}	61.8_{a}	15.1_{a}
		K deficient	0.95_{b}	0.25_{C}	0.10_{b}	0.10_{b}	142_{c}	37.3_{b}	68.5_{a}	10.7_{b}	0.77_{a}	0.07_{b}	0.12_{b}	0.12_{b}	179_{a}	35.4_{b}	23.8_{b}	12.7_{a}
		P deficient	2.20_{a}	0.32_{a}	0.18_{a}	0.18_{a}	434_{a}	72.9_{a}	233_{a}	39.6_{a}	0.80_{a}	0.09_{a}	0.17_{a}	0.14_{a}	321_{b}	42.3_{a}	80.8_{a}	23.2_{a}
		K*P deficient	2.17_{a}	0.32_{a}	0.18_{a}	0.18_{a}	359_{b}	67.3_{a}	80.8_{a}	30.0_{a}	0.84_{a}	0.08_{a}	0.17_{a}	0.14_{a}	425_{a}	43.9_{a}	39.1_{b}	27.7_{a}
	Mean		*1.56_{A}*	*0.30_{A}*	*0.14_{A}*	*0.14_{A}*	272_{A}	53.8_{A}	109_{A}	22.9_{A}	*0.81_{B}*	*0.08_{A}*	*0.14_{A}*	*0.13_{B}*	*293_{A}*	*39.5_{A}*	*51.4_{A}*	*19.7_{A}*
	Significance: $LSD_{5\%}$ (P value[2])																	
	Exp		**	ns	ns	ns	**	ns	*	***	ns	**	*	ns	ns	ns	ns	ns
	CO2 level (CO2)		ns	***	ns	ns	**	ns	ns	ns	*	ns	ns	***	ns	ns	ns	ns
	Fertiliser supply		***	***	***	***	***	***	ns	***	ns	**	***	***	***	***	**	ns
	Exp* CO2		ns	**	**	***	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Exp*Fert.rate	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns
CO2*Fert rate	ns	ns	*	ns	ns	ns	***	***	*	*	ns	ns	ns	ns	ns	ns
Exp* CO2*Fert rate	ns	**	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns

[1]Means with letter subscripts are compared within each column, and those with the same letters not different at α = 0.05.[2] * P<0.05, ** P<0.01, *** P<0.001 and ns = not significant

Appendix 6.2: Grain concentration (N, S, Ca, Mg, Fe, Mn, Zn and Cu) and accumulation for wheat (cv. Discovery) grown with deficient P and K fertiliser supply (Fert.) at elevated carbon-dioxide (eCO_2) in Experiments 3 and 4, at Lincoln, New Zealand in 2019-20 growing season.

			Grain nutrient concentration[1]								Grain nutrient accumulation							
Exp	Treatment		N	S	Ca	Mg	Fe	Mn	Zn	Cu	N	S	Ca	Mg	Fe	Mn	Zn	Cu
	CO2 level	Fert. rate	%				mg/kg DM				mg/tube				mg/tube			
3	Ambient	Control	1.72_b	0.07_b	0.04_a	0.13_a	43.3_b	24.9_a	19.7_b	5.10_b	534_a	23.2_a	12.3_a	40.7_a	3.78_a	0.78_a	0.54_a	0.16_a
		K deficient	1.71_b	0.07_b	0.04_a	0.12_a	50.5_a	25.6_a	17.0_b	4.75_b	486_b	20.7_b	12.3_a	34.6_b	3.61_a	0.73_a	0.50_a	0.14_a
		P deficient	2.34_a	0.08_a	0.04_a	0.10_b	51.7_a	26.5_a	32.4_a	13.5_a	55.9_c	1.85_c	1.03_b	2.48_c	0.62_b	0.06_b	0.09_b	0.03_b
		K*P deficient	2.38_a	0.08_a	0.04_a	0.11_b	60.8_a	26.1_a	32.4_a	6.30_b	43.3_c	1.42_c	0.82_b	1.92_c	0.39_b	0.05_b	0.22_a	0.03_a
	Mean		*2.04_A*	*0.08_A*	*0.04_A*	*0.12_A*	51.6_B	25.8_A	24.9_B	7.50_A	*280_A*	*11.8_A*	*6.6_B*	*19.9_A*	*2.10_B*	*0.40_B*	*0.34_A*	*0.09_A*
	Elevated	Control	1.53_c	0.07_b	0.04_a	0.13_a	63.4_c	28.6_a	18.6_b	3.21_b	540_a	26.0_a	14.0_a	44.7_a	9.89_a	1.01_a	0.60_b	0.11_a
		K deficient	1.65_b	0.07_b	0.04_a	0.11_b	59.6_c	24.4_b	16.1_a	3.18_b	516_b	21.4_b	13.2_b	34.9_b	7.98_a	0.77_b	1.15_a	0.10_a
		P deficient	2.41_a	0.08_a	0.05_b	0.11_a	97.0_b	29.4_a	41.9_a	7.98_a	62.5_c	2.06_c	1.24_c	2.93_c	0.58_b	0.07_c	0.32_c	0.03_b
		K*P deficient	2.38_a	0.08_a	0.05_b	0.11_a	112_a	30.5_a	37.6_a	9.74_a	69.3_c	2.24_c	1.40_c	3.24_c	0.61_b	0.09_a	0.16_c	0.04_b
	Mean		*1.99_A*	*0.07_B*	*0.04_A*	*0.12_A*	*83.1_A*	*28.2_A*	*26.9_A*	*5.62_A*	*297_B*	*12.9_A*	*7.5_A*	*21.5_A*	*4.76_A*	*0.48_A*	*0.56_A*	*0.07_A*
4	Ambient	Control	2.01_b	0.07_b	0.04_a	0.11_b	42.5_b	24.2_b	19.9_b	5.22_a	488_a	21.3_a	10.8_b	36.1_a	8.14_a	0.70_a	0.69_a	0.15_a
		K deficient	1.70_c	0.07_b	0.04_a	0.13_a	36.3_b	23.4_b	25.0_b	3.06_b	481_a	18.6_b	11.5_a	32.1_b	4.29_b	0.63_a	0.62_a	0.08_b
		P deficient	1.78_c	0.07_b	0.04_a	0.12_a	54.6_a	28.4_a	38.9_a	7.29_a	36.5_b	1.21_c	0.69_c	1.70_c	0.45_c	0.05_b	0.10_b	0.02_c
		K*P deficient	2.27_a	0.08_a	0.04_a	0.11_b	47.1_a	26.7_a	31.6_b	6.36_a	46.1_a	1.50_c	0.90_c	2.10_c	0.41_c	0.05_b	0.13_a	0.01_c
	Mean		*1.94_A*	*0.07_A*	*0.04_A*	*0.12_A*	45.2_B	25.7_A	28.9_A	5.36_A	*263_B*	*10.7_A*	*6.0_A*	*18.0_A*	*3.32_B*	*0.36_B*	*0.38_A*	*0.07_A*
	Elevated	Control	1.92_b	0.07_a	0.03_a	0.10_a	35.5_b	24.0_b	16.3_b	2.50_b	510_a	20.8_a	8.20_a	30.6_a	9.96_a	0.74_a	1.86_a	0.08_a
		K deficient	1.67_a	0.07_a	0.03_a	0.10_a	37.8_b	25.0_b	15.3_b	3.09_a	457_b	17.8_b	8.20_a	30.9_a	6.57_b	0.73_a	0.70_b	0.09_a
		P deficient	1.56_a	0.06_b	0.03_a	0.11_a	69.1_a	27.7_a	36.9_a	8.45_a	45.9_d	1.40_c	0.63_b	2.05_b	0.59_c	0.06_a	0.18_c	0.02_b
		K*P deficient	2.24_a	0.07_a	0.03_a	0.10_a	67.0_a	23.1_a	42.0_a	7.55_a	70.7_c	2.12_a	1.02_a	3.12_b	0.59_a	0.08_a	0.27_c	0.02_b
	Mean		1.85_A	0.07_A	0.03_B	0.10_B	52.3_A	25.0_A	27.6_A	5.40_A	271_A	10.5_A	4.50_B	16.7_A	4.43_A	0.40_A	0.75_A	0.05_A
	Significance: $LSD_{5\%}$ (P value[2])																	
	Exp		**	ns	ns	ns	**	ns	*	***	**	***	***	***	ns	**	ns	*
	CO_2 level		ns	***	***	***	**	ns	ns	ns	*	ns	*	ns	*	**	ns	ns
	Fertiliser supply		***	***	***	***	***	***	*	***	***	***	***	***	***	***	**	***

Exp* CO_2	ns	**	**	***	*	ns	ns	ns	ns	ns	***	*	ns	ns	ns	ns
Exp*Fert. supply	ns	ns	ns	ns	ns	ns	**	ns	ns	**	***	***	ns	*	ns	ns
CO_2*Fert. supply	ns	ns	*	ns	ns	ns	***	***	ns	ns	**	ns	ns	ns	ns	ns
Exp* CO_2*Fert. supply	ns	**	ns	ns	ns	ns	*	ns	ns	ns	***	*	ns	ns	ns	ns

[1]Means with letter subscripts are compared within each column, and those with the same letters not different at $\alpha = 0.05$.[2] * $P<0.05$, ** $P<0.01$, *** $P<0.001$ and ns = not significant

Appendix 6.3: Shoot nutrient accumulation (S, Ca, Mg, Fe, Mn, Zn and Cu) at anthesis and harvest maturity for wheat (cv. Discovery) grown with deficient P and K fertiliser supply (Fert.) at elevated carbon-dioxide (eCO_2) in Experiments 3 and 4, at Lincoln, New Zealand in 2019-20 growing season.

			Anthesis							Harvest maturity						
Exp	Treatment		S	Ca	Mg	Fe	Mn	Zn	Cu	S	Ca	Mg	Fe	Mn	Zn	Cu
	CO2 level	Fert. rate	mg/tube							mg/tube						
3	Ambient	Control	97.5_{a}	40.0_{a}	30.2_{a}	2.73_{a}	1.16_{a}	3.21_{a}	0.14_{b}	52.4_{a}	60.2_{b}	78.7_{a}	7.15_{a}	1.51_{a}	1.03_{a}	0.58_{a}
		K deficient	69.9_{b}	40.4_{a}	30.0_{a}	2.47_{a}	0.89_{b}	3.07_{a}	0.12_{b}	43.8_{b}	69.0_{a}	77.1_{a}	6.84_{a}	1.56_{a}	0.94_{a}	0.54_{a}
		P deficient	13.5_{c}	11.1_{b}	8.25_{b}	1.25_{b}	0.29_{c}	0.22_{b}	0.11_{b}	5.38_{c}	13.3_{c}	11.1_{b}	1.69_{b}	0.29_{b}	0.25_{c}	0.10_{b}
		K*P deficient	11.1_{a}	11.4_{b}	7.39_{a}	1.21_{b}	0.25_{a}	0.90_{b}	0.19_{a}	4.63_{c}	13.1_{c}	9.74_{b}	1.10_{b}	0.24_{b}	0.68_{b}	0.10_{b}
	Mean		*48.0_{B}*	*25.7_{B}*	*19.0_{B}*	*1.91_{B}*	*0.65_{B}*	*1.85_{A}*	*0.14_{B}*	*26.6_{B}*	*38.9_{B}*	*44.2_{A}*	*4.20_{B}*	*0.90_{B}*	*0.72_{A}*	*0.33_{B}*
	Elevated	Control	153_{a}	69.8_{b}	42.6_{b}	6.72_{a}	1.73_{a}	3.34_{a}	0.19_{a}	58.5_{a}	61.8_{b}	81.1_{a}	11.0_{a}	2.36_{a}	1.23_{a}	0.86_{a}
		K deficient	118_{a}	76.5_{a}	49.5_{a}	6.04_{a}	1.38_{b}	2.79_{a}	0.22_{a}	42.9_{b}	81.5_{a}	84.0_{a}	11.9_{a}	2.23_{a}	0.96_{a}	0.79_{a}
		P deficient	12.0_{b}	10.8_{c}	7.40_{c}	1.32_{b}	0.24_{c}	0.60_{b}	0.15_{b}	5.57_{c}	13.1_{c}	11.3_{b}	7.91_{b}	0.33_{b}	0.85_{a}	0.15_{b}
		K*P deficient	11.4_{b}	10.6_{c}	7.39_{c}	1.35_{b}	0.24_{c}	0.90_{b}	0.12_{b}	7.15_{c}	18.4_{c}	14.2_{b}	3.97_{c}	0.39_{b}	0.45_{b}	0.21_{b}
	Mean		*73.4_{A}*	*41.9_{A}*	*26.7_{A}*	*3.86_{A}*	*0.90_{A}*	*1.91_{A}*	*0.17_{A}*	*30.0_{A}*	*43.7_{A}*	*47.7_{A}*	*8.69_{A}*	*1.33_{A}*	*0.87_{A}*	*0.50_{A}*
4	Ambient	Control	91.1_{a}	36.7_{b}	27.8_{b}	4.30_{a}	1.02_{a}	1.27_{a}	1.27_{b}	46.8_{a}	52.7_{b}	71.6_{b}	11.2_{a}	1.48_{b}	1.34_{a}	0.68_{a}
		K deficient	69.4_{b}	40.3_{a}	29.8_{a}	4.58_{a}	0.90_{a}	1.07_{a}	1.34_{a}	37.2_{b}	69.2_{a}	77.2_{a}	6.24_{b}	1.83_{a}	1.15_{a}	0.54_{a}
		P deficient	10.7_{c}	9.60_{d}	7.31_{c}	1.15_{b}	0.25_{b}	0.33_{b}	0.41_{c}	3.15_{c}	7.33_{c}	6.95_{b}	4.21_{b}	0.24_{c}	0.28_{b}	0.26_{b}
		K*P deficient	13.2_{c}	13.2_{c}	9.7_{c}	1.87_{b}	0.28_{b}	0.37_{b}	0.71_{c}	4.70_{c}	13.8_{c}	11.4_{b}	5.50_{b}	0.31_{c}	0.35_{a}	0.10_{b}
	Mean		*46.1_{B}*	*25.0_{B}*	*18.6_{B}*	*2.98_{B}*	*0.61_{B}*	*1.19_{A}*	*3.73_{B}*	*24.2_{B}*	*37.2_{B}*	*43.8_{A}*	*6.90_{B}*	*1.01_{B}*	*0.81_{A}*	*0.42_{B}*
	Elevated	Control	112_{a}	47.9_{b}	33.3_{b}	5.89_{a}	1.46_{a}	2.19_{a}	1.72_{a}	50.8_{a}	66.9_{a}	62.8_{b}	15.4_{a}	2.25_{a}	1.36_{a}	0.93_{a}
		K deficient	90.7_{b}	52.3_{a}	37.0_{a}	5.11_{b}	1.34_{a}	2.47_{a}	1.54_{b}	39.9_{b}	69.2_{a}	73.5_{a}	9.37_{b}	2.11_{a}	1.38_{a}	0.75_{b}
		P deficient	13.3_{c}	11.1_{c}	7.37_{c}	1.79_{c}	0.30_{b}	0.92_{b}	0.73_{c}	4.94_{c}	10.1_{b}	8.31_{b}	4.50_{c}	0.25_{b}	0.49_{b}	0.13_{b}
		K*P deficient	12.0_{a}	10.4_{c}	6.90_{a}	1.36_{c}	0.25_{b}	0.31_{b}	0.45_{c}	6.50_{c}	14.7_{a}	11.9_{b}	11.5_{b}	0.36_{b}	0.69_{b}	0.23_{b}
	Mean		*57.1_{A}*	*30.4_{A}*	*21.2_{A}*	*3.54_{A}*	*0.84_{A}*	*1.47_{A}*	*4.45_{A}*	*25.6_{A}*	*40.2_{A}*	*39.1_{A}*	*10.2_{B}*	*1.24_{A}*	*0.96_{A}*	*0.51_{A}*
	Significance: $LSD_{5\%}$ (P value[2])															
	Exp		***	***	***	ns	ns	*	***	**	ns	**	ns	ns	ns	ns
	CO2 level (CO2)		***	***	***	***	***	ns	**	**	*	ns	**	***	ns	**

Fertiliser supply	***	***	***	***	***	*	***	***	***	***	***	***	**	***
Exp* CO2	***	**	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Exp*Fert. supply	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CO2*Fert. supply	***	***	***	***	***	ns	ns	ns	ns	ns	ns	ns	ns	ns
Exp* CO2*Fert. supply	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[1]Means with letter subscripts are compared within each column, and those with the same letters not different at $\alpha = 0.05$.[2] * $P<0.05$, ** $P<0.01$, *** $P<0.001$ and ns = not significant